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MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

XLVI SEPTEMBER–OCTOBER, 1954 No. 5

THE PRODUCTION OF ETHYLENE BY PENICILLIUM DIGITATUM [1]

CHARLES L. FERGUS

(WITH 12 FIGURES) (5 CUTS)

A number of green plant tissues, including fruits, produce physiologically active emanations which cause epinastic responses of leaves, the "triple response" of etiolated pea seedlings, the inhibition of seed germination, and also the acceleration of the rate of respiration and softening of fruits. These emanations have been proven to be ethylene.

The production of a similar substance by fungi has not been extensively investigated. Miller *et al* (**5**) were the first to report that emanations from pure cultures of *Penicillium digitatum* Sacc. produced epinasty of potato plants. Biale and Shepherd (**1**) reported that when *P. digitatum* was grown on potato dextrose agar, it produced emanations which induced epinasty of pea seedlings and also accelerated the respiration and ripening of lemon fruits, but it did not produce them when grown on a synthetic medium.

No chemical proof that the fungus emanation is ethylene has as yet been offered. Thorough investigation of a large number of volatile chemical compounds has revealed that only ethylene (0.2 ppm), carbon

[1] Based on a portion of a thesis submitted to the graduate school of the Pennsylvania State College in June, 1948, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Appreciation is hereby expressed by the author to Dr. J. W. Sinden for his advice and encouragement during the course of the work.

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monoxide (5000 ppm), acetylene (250 ppm) and propylene (1000 ppm) possess such an ability (**3**). Of these, ethylene is the only one arising from biological processes, and since all but ethylene are required in large concentrations, it appears logical to assume that the active emanation from *P. digitatum* is ethylene.

The ethylene production of *P. digitatum* was studied for the purpose of clarifying its method of origin and its relation to growth and metabolism.

MATERIALS AND METHODS

The organism used in this study was *P. digitatum* strain NRRL No. *1202.*

The "triple response" of etiolated Alaska pea seedlings, prepared and grown according to the method of Pratt and Biale (**7**), was used to determine the amounts of ethylene produced. The "triple response" consists of inhibition of epicotyl elongation, declination from the vertical, and swelling of epicotyl tips.

Photographs of a standard set of seedlings, i.e., seedlings exposed to known concentrations of ethylene in air (FIG. 1), served as comparators

FIG. 1. The response of etiolated pea seedlings to known concentrations of ethylene gas in air. Top (from left): no ethylene, 0.25 and 0.5 parts per million. Bottom: 1.0, 2.5, and 5.0 parts per million.

throughout the study to determine the quantity of ethylene produced by the fungus in the different tests.

In conducting a test for ethylene production, the culture flask was placed on a glass plate after removing the cotton plug. A petri dish of test seedlings was also placed on the glass plate. A bell jar of 5750 ml volume was then placed over the flask and petri dish, and an air-tight seal made with "Litholine." Controls were always obtained under identical conditions, the only difference being that no fungus culture was placed under the bell jar with the seedlings. After the seedlings were exposed 48 hours under the bell jar in the dark, the physiological response obtained was determined by comparison with the photographs of the "standard seedlings," since the response of the seedlings is the same for the same concentration of ethylene under comparable conditions at any time. The results are presented as parts of ethylene per million parts of air in bell jars of 5750 ml capacity.

Methods of inoculation, incubation and harvesting of mycelium, and the basal synthetic medium used were described in a previous publication, Fergus (**4**). Ethylene production in both shaken and unshaken cultures was studied.

In the series of experiments in which ethylene production from specific metabolites was studied, the experimental procedure was varied to avoid any possible toxic effect of the metabolite on spore germination. After the fungus had grown for 16 days on small amounts of glucose, the metabolites were added singly in equivalent amounts at one time or in small amounts every third day. After 12 days' growth, mycelial weights and ethylene production were determined.

Each value reported is the average of three replicates. When ethylene production was determined over a long period of time, each value presented is the average of the response (three replicates) obtained at one specific time interval.

EXPERIMENTAL RESULTS

A. THE EFFECT OF MECHANICAL SHAKING. From the information gained in the nutrition studies of *P. digitatum* (Fergus, **4**) it appeared possible to study ethylene production under various nutritional conditions, speeding up growth by shaking, thus making it possible to obtain more data over a given period of time than would be possible if unshaken cultures were studied.

P. digitatum was grown on the shaker in the basal synthetic medium with no added growth factors for seven days. Bell jars were firmly fixed on top of the shaker and tests for ethylene production were carried

out under continuous shaking. Positive epinastic responses were obtained. The shaking had no injurious effect on the test seedlings for they grew as straight as they do when not shaken. The interlacing and interweaving of the roots form a strong hold, and the shaking did not knock the seedlings over.

Because of the lack of space on the shaker, later experiments were performed by removing the flasks from the shaker after the desired time period and placing them under a bell jar on a glass plate in a stationary position for the ethylene determination.

TABLE I

PRODUCTION OF ETHYLENE BY P. DIGITATUM ON DIFFERENT CARBON * SOURCES

Compound	Days growth	Economic** coefficient	Ethylene ppm	Compound	Days growth	Economic** coefficient	Ethylene ppm
D-xylose	11	.355	5.0	Dextrin	11	.057	0
L-Arabinose	11	.331	1.0	Dextrin	16	.127	.5
D-Glucose	11	.349	.5	Starch	16	0	0
D-Fructose	11	.377	2.5	Citric acid	11	.092	5.0
D-Mannose	11	.375	10.0	Fumaric acid	11	0	0
D-Galactose	11	.344	5.0	Succinic acid	11	.008	1.0
L-Rhamnose	16	.227	1.0	Oxalic acid	11	0	0
D-Mannitol	11	.061	10.0	Oxalic acid	16	0	0
Sucrose	11	.320	1.0	Lactic acid	11	0	0
Sucrose	16	.346	2.5	Lactic acid	16	0	0
Maltose	11	.074	1.0	Acetic acid	11	0	0
Maltose	16	.091	0	Acetic acid	16	0	0
Lactose	11	.260	.5	L-Malic acid	11	.072	1.0
Lactose	16	.312	0	Tartaric acid	11	0	0
Cellobiose	11	.353	.5	Tartaric acid	16	0	0
Inulin	11	0	0	Peptone	11	.105	.5
Inulin	16	0	0	Casein	11	.124	.5

* Basal medium. Shaken. Each flask contained 0.5410 gram of carbon. The pH of each medium was adjusted to 4.4.

** Economic coefficient is mg carbon in mycelium (assumed to be 50 percent carbon, see Raistrick *et al*, **8**) divided by mg carbon supplied by substrate. Each value is based on three replicates.

B. THE RELATION OF NITROGEN SOURCE TO ETHYLENE PRODUCTION. In the experiments concerning the utilization of different nitrogen compounds by *P. digitatum* (Fergus, **4**) (in the basal medium with no added growth factors) tests for ethylene production were made after 11 days' growth. In all cases, a positive epinasty of the test seedlings was obtained.

From these observations it was concluded that if the nitrogen source could be utilized for growth by the fungus, ethylene production also occurred.

C. THE RELATION OF CARBON SOURCE TO ETHYLENE PRODUCTION. Biale and Shepherd (**1**) found that *P. digitatum* did not produce ethylene when grown in a synthetic medium containing glucose and various mineral salts. Pratt (**6**) reported that when sucrose was present in a synthetic medium, the fungus produced ethylene. These results suggested the possibility that *P. digitaum* produces ethylene only when specific nutrients are present. Therefore, a large number of organic compounds were tested to determine whether *P. digitatum* produces ethylene from a few specific compounds, or if any carbon source will allow its production.

The results are presented in TABLE I. *P. digitatum* produced ethylene in all of the organic substrates in which it could grow. The amount of

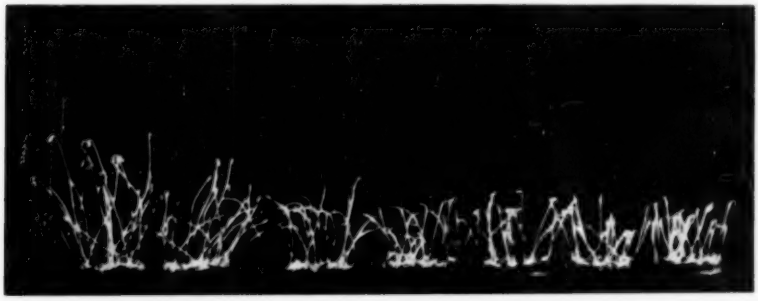


FIG. 2. The response of etiolated pea seedling to the emanations of *P. digitatum*. From the left: no emanations, two replicates each when glucose, sucrose, and xylose were carbon sources.

mycelium did not determine the quantitative production of ethylene since the maximum amount was not always obtained in the media which allowed maximum mycelial production. Photographs of the responses of the pea seedlings to the emanations of *P. digitatum* when the carbon sources were D-glucose, sucrose and D-xylose are presented in FIG. 2.

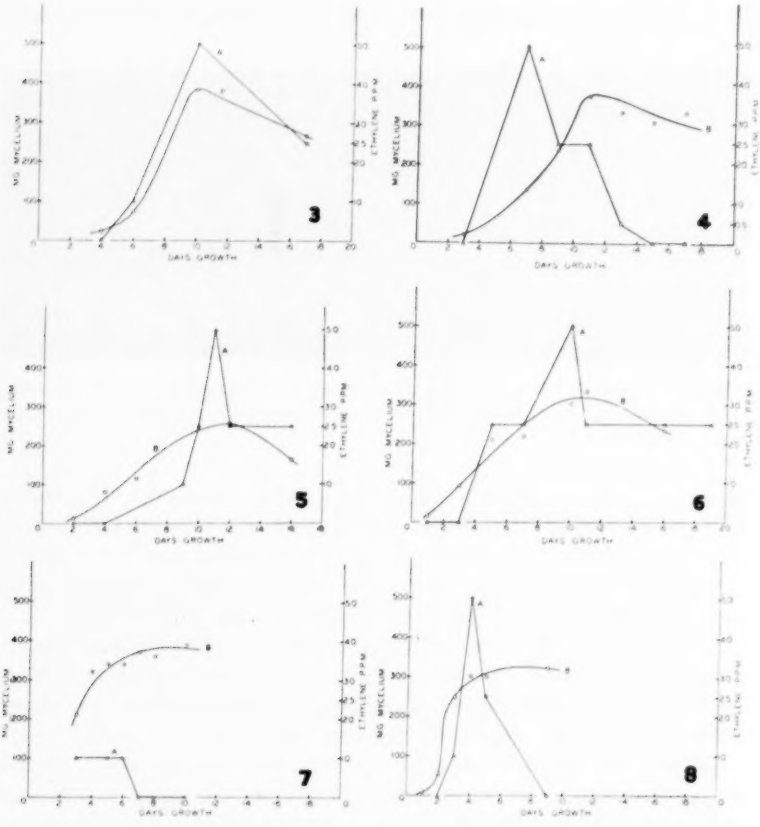
In some cases a positive result was obtained at one sampling date, and not at another on the same compound. This may be due to different lengths of time before ethylene production begins and ends on different substrates, or it may be due to biological variation.

D. THE RELATION OF GROWTH TO ETHYLENE PRODUCTION. The production of ethylene by *P. digitatum* in the basal medium was followed over relatively long periods of time. In those cases in which the only response of the test seedlings obtained was one of inhibition of elongation of the epicotyl, the production was considered as zero since this

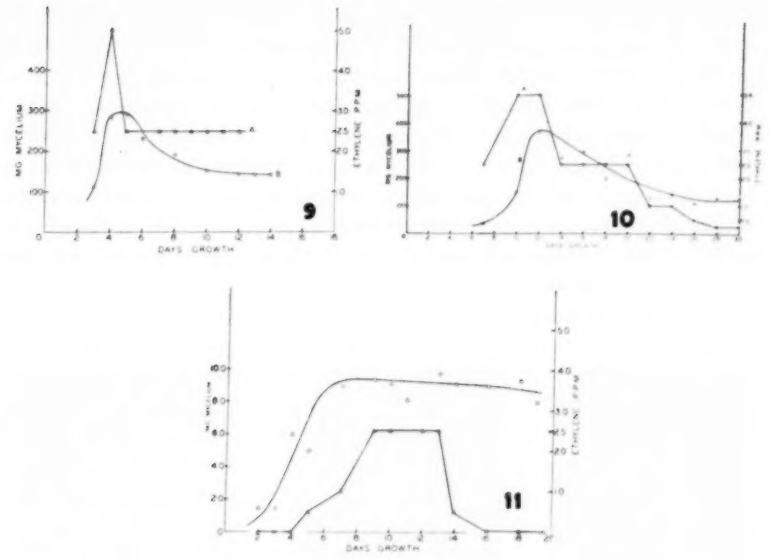
shortening effect might well be due to the alcohol produced by the fungus. (See later.)

FIGS. 3 and 4 depict growth and ethylene production in the basal medium in unshaken flasks. The maximum production of ethylene occurred simultaneously or just prior to maximum mycelial production. The duration of ethylene production was not the same in the two experiments. The reason for this is not known, since the time-growth curves in both experiments agreed rather well.

FIGS. 5 and 6 show the results obtained when *P. digitatum* was grown in the basal medium with vitamins added. The vitamins were: 0.02



FIGS. 3–8. Ethylene production, A, and growth, B. 3, 4. In the basal medium, unshaken flasks. 5, 6. In the basal medium with vitamin mixture added, unshaken flasks. 7, 8. In the basal medium with vitamin mixture added, flasks shaken.



FIGS. 9–11. Ethylene production, A, and growth, B. 9. In the Wooster and Cheldelin medium, unshaken flasks. 10. In the basal medium with 10 gm potassium acetate added, flasks shaken. 11. Growth, upper curve, and ethylene production, lower curve, in the basal medium with 1 gm glucose substituted for sucrose, flasks shaken.

gamma biotin, 30 gammas calcium pantothenate, 40 gammas pyridoxine hydrochloride and 20 gammas thiamin hydrochloride per flask. The flasks were not shaken. In both of these experiments maximum ethylene production occurred shortly before or simultaneously with maximum mycelial production. Again, the period of ethylene production was variable.

FIGS. 7 and 8 show the effect of shaking on ethylene production when the basal medium contained vitamins. The general effect of shaking on ethylene production was to inhibit or shorten the duration of the production. Ethylene production occurred just prior to or simultaneously with maximum mycelial production in both of these experiments. In the experiment depicted in FIG. 7 the maximum mycelial production was greater but ethylene production was less than that depicted in FIG. 8. The reason for this discrepancy is not known.

E. THE RELATION OF pH CHANGES TO ETHYLENE PRODUCTION. When *P. digitatum* is grown in the basal medium the pH drops pro-

gressively from the initial pH 4.4 to 2.2 (Fergus **4**, Fig. 1). However, when the fungus is grown in the Wooster and Cheldelin medium, the pH rises from the initial pH 3.0 to 7.4 with a subsequent drop (Fergus **4**, Fig. 10). The possibility existed that these pH differences might affect ethylene production; therefore, growth and ethylene production were studied in the Wooster and Cheldelin medium in unshaken flasks (see Fergus, **4**, for its composition) and the basal medium to which 10 gm of potassium acetate were added for buffering effect (in shaken flasks). The time-growth curves and ethylene production in these two media are presented in FIGS. 9 and 10.

In the Wooster and Cheldelin medium (FIG. 9), maximum ethylene production occurred just prior to maximum mycelial production and although autolysis occurred, ethylene was produced for the duration of the experiment. The time-growth curve obtained compares favorably with the ones obtained previously (Fergus, **4**, Figs. 9 and 10), and the pH values were also found to be similar.

The time-growth curve obtained in the basal-potassium acetate medium (FIG. 10) is similar to the type obtained when an organic nitrogen source is present in the medium, such as the Wooster and Cheldelin medium, except that there is a long lag period before maximum growth occurs. These results are similar to those obtained by Camp (**2**) who found that heavy buffering with potassium citrate delayed growth initiation, although the ultimate rate and maximum amount of growth were not affected.

F. THE PRODUCTION OF ETHYLENE FROM SPECIFIC METABOLITES. In the metabolism of many fungi ethanol and various acids are formed as the result of incomplete oxidation of the substrate. Many of these form in very small amounts or are only transitory in nature, being converted to other compounds by oxidation, dehydrogenation or decarboxylation. The ability of *P. digitatum* to produce ethylene when any of these specific metabolites are available might yield information helpful in postulating the origin of ethylene in the metabolic cycle. Therefore, various acids and ethanol were tested as substrates.

Prior to testing the ability of *P. digitatum* to produce ethylene from ethanol, the effect of ethanol on pea seedlings was determined. Petri dishes of seedlings were placed under bell jars of 5.7 liters capacity containing a beaker of 50 ml of water or alcohol solution, ranging in concentration from 0.5 to 15 percent (by volume).

The alcohol vapor caused an inhibition of elongation of epicotyls at 3.5 percent, and at higher concentrations the inhibition gradually became greater. A slight thickening of epicotyls resulted when the seedlings

were exposed to 15 percent ethanol. Photographs of the responses of seedlings to ethanol are presented in FIG. 12.

These results indicate that the pea seedling test is not useful to determine certain concentrations of ethylene if ethanol is also present, for from the range of 0.05 to 0.15 ppm, the only response of the seedling to ethylene is a shortening of epicotyls. In growing *P. digitatum* the odor of ethanol was frequently noticed, particularly in stationary cultures. The effect of the ethanol produced by *P. digitatum* on test seedlings was determined by filtering the mycelium out of culture flasks in which the fungus had grown for 12 days, and exposing seedlings to the filtrates. Shortening of epicotyls, but no thickening was caused by the filtrates.

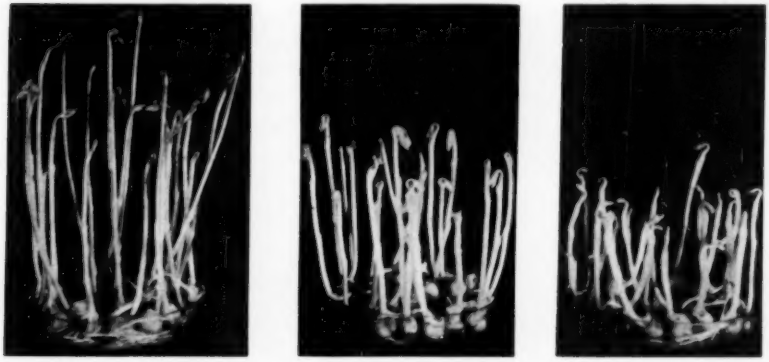


FIG. 12. The response of etiolated pea seedlings exposed to ethanol vapor. From left: no alcohol, 3.5 and 15 percent.

Therefore, only the range from 0.25 to 10.0 ppm of the standard seedlings set was used in the investigation.

The method described under Materials and Methods, based on the following preliminary experiment, was used to test ethylene production from various metabolites. *P. digitatum* was grown in the basal medium with 1 gm of glucose per liter substituted for the 25.7 gm sucrose. Mycelial dry weight determinations, reducing sugar tests of the medium, and ethylene tests were made a frequent intervals. Maximum production of mycelium, FIG. 11, occurred after 8 days' growth, negative reducing sugar tests were obtained from the 12th day on, and ethylene production ceased after the 14th day, FIG. 11. After the 16th day, 0.05 gm glucose was added aseptically to each flask. Five days later positive ethylene tests were obtained, and the dry mycelial weight had increased to 22 mg.

When the metabolites (with the exception of fumaric acid) were added to the media in small amounts at frequent intervals after the initial 1 gm glucose had been utilized, the resultant growth of *P. digitatum* was sufficient for the production of ethylene on the basis of a production of 9 mg mycelium in the glucose medium (see FIG. 11). The fungus produced ethylene from lactic, citric, L-malic and succinic acids, but did not produce it from ethanol, acetic, pyruvic and fumaric acids. Because of the importance of pyruvic acid in the schemes of carbohydrate dissimilation, the negative ethylene result obtained when this acid was the carbon source, and 32 mg of mycelium were produced, was not expected.

TABLE II

EFFECT OF METHOD OF SUBSTRATE ADDITION * ON THE EFFICIENCY OF P. DIGITATUM TO UTILIZE SUBSTRATES EXPRESSED AS ECONOMIC COEFFICIENTS ** AND UPON ETHYLENE PRODUCTION

Substrate	Added at one time	Ethylene*** ppm	Small amounts added at various intervals	Ethylene ppm
Acetic acid	0	0	.206	0
Lactic acid	.140	.5	.144	.5
Citric acid	.076	2.5	.134	1.0
Pyruvic acid	0	0	.056	0
L-Malic acid	.060	0	.101	0.5
Succinic acid	.061	0.5	.091	1.0
Glucose control	.500	2.5	—	—

* Substrate added 4 days after negative reducing sugar and negative ethylene tests were obtained.
** See footnote 2, TABLE I.
*** Ethylene test made 12 days after initial substrate addition.

At low pH values, organic acids exist predominantly as free acid and in this state may be quite toxic. The method of addition of small amounts at various time intervals to small amounts of preformed mycelium may be important in the elucidation of the utilization of different metabolites which are toxic in larger amounts, either due to a pH effect or a toxic effect on spore germination, TABLE II. The result obtained with acetic acid is the most striking example.

DISCUSSION

The data obtained in the course of this investigation show that the production of the physiologically active emanation, presumably ethylene, is a definite part of the metabolism of *P. digitatum*. If chance governed the production, there would be no well-defined trend. This has not been found to be the case. On the contrary, the production of

ethylene under a variety of cultural conditions, follows a regular course. As the fungus begins active growth, small amounts of ethylene are produced, and as growth progresses there is an increase to a maximum, which occurs prior to or simultaneously with maximum mycelial production. This maximum is followed by a gradual decline. However, since ethylene production continued in many of the experiments long after autolysis had commenced, it would appear that its production is not related directly to an assimilatory activity.

The relationship of ethylene production to respiration of *P. digitatum* should be studied. Pratt (**6**) on the basis of a single microrespiration study suggested that ethylene production is closely related to respiration. He measured oxygen uptake only, and yet anaerobic respiration is important in the catabolism of *P. digitatum* as indicated by the results of various workers, and also by the fact that in this investigation, the odor of ethanol was detected often. It was especially strong in unshaken flasks. The relation of respiration to ethylene production should be studied by simultaneously determining oxygen uptake, carbon dioxide evolution and ethanol and ethylene production. The relationship might then be clarified.

The possibility exists that there is no direct relationship between ethylene production and respiration. This is suggested by the fact that, in several experiments reported herein, ethylene production continued for a considerable period of time at a relatively high level, after considerable autolysis had occurred. It seems logical to suppose that the respiration of autolyzing cultures is relatively small.

Ethylene production in shaken flasks was usually more erratic and of lesser magnitude than in stationary flasks. There are two possible explanations for this result. One, shaking may have increased the rate of diffusion of ethylene from the flasks into the air through the cotton stoppers prior to the seedling test. And two, shaking may have affected the metabolism of the fungus so that actually less ethylene was produced.

SUMMARY

P. digitatum does not require any specific nitrogen source to produce ethylene. If organic and inorganic nitrogen compounds allowed growth, ethylene was produced. No specific carbon source is necessary for ethylene production, for when a large number of organic compounds were substituted singly in the basal medium, the fungus produced ethylene.

A method was devised to avoid possible toxic effects of a compound on *P. digitatum* in order to study ethylene production when ethanol

and various acids were added to the medium. Small amounts of the compounds were added at several intervals after growth had become established on small amounts of glucose, but after all the glucose had disappeared from the medium. A considerable amount of growth occurred on acetic and pyruvic acid compared to no growth when the acids were added at the start. Citric, malic, lactic and succinic acids allowed ethylene production whereas acetic, pyruvic and fumaric acids, and ethanol did not. Growth was poor on the latter compounds, but enough mycelium was formed to allow for production of ethylene based on the production of 2.5 ppm from 9 mg mycelium in 0.1 percent glucose medium. An exception was fumaric acid, which was not utilized by *P. digitatum*.

Low concentrations of ethanol vapor inhibited elongation of pea seedlings and also caused leaf burn. When the fungus was grown in the basal medium in stationary flasks, ethanol was produced in quantities which inhibited elongation of the epicotyls of etiolated pea seedlings.

The production of ethylene by *P. digitatum* follows a regular course under a variety of different cultural conditions. Small amounts are produced as the fungus begins active growth, and this is followed by a rise to a maximum with subsequent reduction. The maximum production of ethylene occurred just prior to or simultaneously with maximum mycelial production.

There does not appear to be any direct relationship between the amount of mycelium formed and the amount of ethylene produced.

The addition of vitamins to the basal medium is not required for ethylene production. However, their addition invokes earlier initial ethylene production. This is accompanied by an acceleration of the initial growth of the fungus.

The effect of shaking on ethylene production of *P. digitatum* was to cause variable production. In some cases complete inhibition of ethylene production was observed, and in other cases the duration of production and the amount of ethylene produced was reduced.

Ethylene was produced at both high and low residual pH values.

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LITERATURE CITED

1. **Biale, J. B. and A. Shepherd.** 1941. Respiration of citrus fruits in relation to metabolism of fungi. I. Effects of emanations of *Penicillium digitatum* Sacc. on lemons. Amer. Jour. Bot. **28**: 263–270.

2. **Camp, A. F.** 1923. Citric acid as a source of carbon for certain citrus fruit-destroying fungi. Ann. Missouri Bot. Garden **10**: 213–298.
3. **Crocker, W.** 1948. Physiological effects of ethylene and other unsaturated carbon-containing gases. In *The Growth of Plants.* Reinhold, N. Y. Pp. 139–171.
4. **Fergus, C. L.** 1952. The nutrition of *Penicillium digitatum* Sacc. Mycologia **44**: 183–199.
5. **Miller, E. V., J. R. Winston and D. F. Fisher.** 1940. Production of epinasty by emanations from normal and decaying citrus fruits and from *Penicillium digitatum.* Jour. Agr. Res. **60**: 269–278.
6. **Pratt, H. K.** 1944. Studies in the physiology of *Penicillium digitatum* Sacc. Ph.D. Thesis. (Unpublished.) Univ. of Calif.
7. —— **and J. B. Biale.** 1944. Relation of the production of an active emanation to respiration in the avocado fruit. Plant Physiol. **19**: 519–528.
8. **Raistrick, H., et al.** 1931. Studies in the biochemistry of microorganisms. Phil. Trans. Roy. Soc. London, Series B **220**: 1–367.

SYNTHESIS OF RIBOFLAVIN BY ASHBYA GOSSYPII GROWN IN A SYNTHETIC MEDIUM

J. J. Goodman and Rosalie Reardon Ferrera

Chemically defined media for the growth of *Ashbya gossypii* (Ashby and Nowell) Guill. have been described by Kögl and Fries (**4**) and by Robbins and Schmidt (**9**) in connection with the use of this organism for the microbiological assay of biotin. In neither case, however, was any mention made of the excretion of riboflavin into the medium. Subsequent investigators (**7**, **8**) do not report any production of riboflavin by known flavogenic strains in synthetic media. This paper reports on modifications in the above synthetic media which have led to production of appreciable amounts of riboflavin.

MATERIALS AND METHODS

The culture studied was *Ashbya gossypii* N.R.R.L. *Y1056* obtained from the Northern Regional Research Laboratory. Agar stocks were kept on Wickerham's M-Y medium (**7**). Two types of media were used to grow the inoculum. Medium for inoculum A was chemically undefined and was prepared as follows: Commercial glucose (Cerelose) 2.0%, Difco Bacto Peptone 0.5%, Corn Steep Liquor 2.5%. The pH was adjusted to 6.8 with KOH before sterilization. Cultures were grown on this medium for 48 hours after which they were used to inoculate the fermentation flasks at the rate of 1% inoculum on a volume basis. With the development of a chemically defined medium which would support riboflavin synthesis, a chemically defined medium for inoculum, referred to as inoculum B was also used. In this case the organism was first grown in inoculum A medium for 24 hours. One percent by volume of this was transferred to flasks of B inoculum medium and allowed to grow for 48 hours before the final transfer was made into the fermentation flasks.

All the inoculum and fermentation cultures were grown at 28–29° C on a reciprocating shaker oscillating at 100 two inch strokes per minute. Inoculum was grown in 25 ml amounts in either 125 or 250 ml Erlenmeyer flasks. One hundred ml of medium per 500 ml Erlenmeyer flasks was used in the fermentation flasks.

Riboflavin was assayed fluorometrically on the acidified culture broths after suitable dilution (**8**). All assays reported are for a 5 day fermentation period.

RESULTS

One starting point for the investigation was the use of glucose-ammonium tartrate-salts medium of Kögl and Fries (**4**) modified by the addition of 40 μg biotin per liter. The second approach was the use of sucrose-asparagine-salts medium of Robbins and Schmidt (**9**) modified by the addition of 0.4 μg biotin per liter.

MEDIUM OF KÖGL AND FRIES: During preliminary trials with the Kögl and Fries medium, both on agar slants and in shaken cultures, it was noted that the addition of DL-Asparagine and Tween 80[1] to the medium resulted in the typical yellow color of ribroflavin. TABLE I presents some typical data on the effect of Tween 80 and asparagine.

Subsequent trials demonstrated that both ammonium lactate and ammonium acetate substituted for the ammonium tartrate in the original formula supported better yields of riboflavin and that the asparagine

TABLE I

EFFECT OF TWEEN 80 AND DL-ASPARAGINE ON RIBOFLAVIN SYNTHESIS IN KÖGL AND FRIES MEDIUM (μG/ML) *

% Tween 80 (vol./vol.)	No asparagine	500 mg/l asparagine
0.0	1	1
0.4	16	14
0.8	14	18
1.0	13	23
1.2	13	22
1.4	14	23
1.8	14	23
2.0	12	26

* Inoculum A

could be omitted from such media. In TABLE II are presented data on the substitution of ammonium acetate for tartrate in the Kögl and Fries medium.

A chemically defined inoculum medium based on the data presented in TABLE II was tested in order to avoid as much as possible a carry-over of undefined nutrients from the corn steep-peptone inoculum. This inoculum medium consisted of the basic Kögl and Fries formula modified by the addition of 50 mg/l of DL-Asparagine and 1% v/v Tween 80 and with the substitution of 2.5 g/l ammonium acetate for the tartrate in the original formula. It is referred to as inoculum B. It was soon found that the use of inoculum B resulted in much lower yields of riboflavin than when inoculum A was used.

A number of experiments were performed to determine whether

[1] Tween 80: Polyoxyethylene sorbitan monooleate. Atlas Powder Company, Wilmington, Delaware.

TABLE II

THE EFFECT OF AMMONIUM ACETATE AND TWEEN 80 ON RIBOFLAVIN SYNTHESIS IN A TARTRATE FREE KÖGL AND FRIES MEDIUM (μG/ML) *

% Tween 80 (vol./vol.)	Ammonium acetate, gm/l			
	0.62	1.25	2.5	5.0
0.0	—	—	13	—
0.05	6	6	55	—
0.1	—	—	70	—
0.3	—	—	93	—
0.4	26	39	114	—
1.0	41	44	120	145

* Inoculum A.

higher riboflavin yields obtained with inoculum A were due to a carry-over of corn steep liquor or peptone. Repeated washing of inoculum A in distilled water before use still resulted in higher yields compared to those obtained with inoculum B. The addition of a calculated carry-over amount of corn steep liquor and peptone to the synthetic medium did not result in higher assays when inoculum B was used. Based on analyses obtained from the manufacturer, the vitamins and amino acids found in corn steep liquor were added in various combinations to the synthetic medium which was then inoculated with inoculum B. The addition of the vitamins did not improve the yields. Amino acids in certain combinations did improve the yields somewhat but not to the same extent as when inoculum A was used without amino acids in the fermentation medium. The results of the addition of amino acids to the medium are presented in TABLE III.

TABLE III

EFFECT OF ADDING AMINO ACIDS TO SYNTHETIC MEDIUM * INOCULATED WITH INOCULUM GROWN IN SYNTHETIC MEDIUM

Amino acids added	Riboflavin μg/ml
None	11
All**	36
Alanine, arginine, glutamic acid, histidine, methionine	42
Alanine, aspartic acid, cystine, lysine, proline	30
Alanine, isoleucine, leucine, phenylalanine, threonine tyrosine, valine	9

* Basal medium: Kögl and Fries medium modified by the additon of 1% Tween 80 and the use of ammonium acetate at 2.5 g/l in place of tartrate.

** Amino acids mg per liter: Alanine 20, arginine 5, DL-aspartic 2, L-cystine 1, DL-glutamic 10, L-histidine 2, DL-Isoleucine 3, D-leucine 5, DL-lysine 2, DL-methionine 2, DL-phenylalanine 3, L-proline 4, DL-threonine 2, tyrosine 1, DL-valine 3.

The data in TABLE III, together with those in which washed inoculum A was used, point to a possible conditioning effect of the chemically undefined inoculum medium conducive to high yields in a synthetic medium. The amount of growth using inoculum A is much greater than when inoculum B was used, and this may have been a factor in inducing higher yields with the former. This was not specifically tested but seems rather unlikely from the following consideration: The Robbins and Schmidt medium (**9**) always resulted in a heavy growth of *A. gossypii*, yet when used as inoculum medium for the synthetic medium

TABLE IV

EFFECT OF ASPARAGINE AND AMMONIUM SULFATE ON RIBOFLAVIN SYNTHESIZED IN A MODIFIED KÖGL AND FRIES MEDIUM (μG/ML)

Material added	Inoculum A	Inoculum B
Basal*	92	19
Basal* + 2 g/l asparagine	45	—
Basal* + 5 g/l asparagine	—	10
Basal* + 2 g/l $(NH_4)_2SO_4$	115	—
Basal* + 5 g/l $(NH_4)_2SO_4$	—	34

* Basal: Original Kögl and Fries medium with the addition of 1% Tween 80 and 2.5 g/l ammonium acetate in place of tartrate.

such growth resulted in only a fraction of the riboflavin synthesized when inoculum A was used.

One further modification of the Kögl and Fries medium was made in the addition of ammonium sulfate to the basic formula. As shown in TABLE IV this resulted in somewhat better yields of riboflavin.

In all cases tested, ammonium sulfate addition resulted in higher final yields. The reason for this effect is not known, but it does not appear to be a result of a more favorable pH level, since no consistent correlation could be found between yield and pH level at harvest in any experiment.

The final modified Kögl and Fries medium was as follows:

Dextrose	20 gm
$K^1H_2PO_4$	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
$NH_4C_2H_3O_2$	2.5 gm
$(NH_4)_2SO_4$	5.0 gm
$CaCl_2$	0.1 gm
NaCl	0.1 gm
$FeCl_3$	trace*
Biotin	0.4 μg
Thiamin·HCl	40 μg
Meso inositol	10 mg
Tween 80	1% vol./vol.
Distilled water	to make one liter

* 3 drops of a 1% solution.

In TABLE V are presented some typical data using the modified medium. It will be seen that regardless of the inoculum used, the original medium permits only slight amounts of riboflavin to be synthesized as compared with the modified medium.

MEDIUM OF ROBBINS AND SCHMIDT: Media based on the Kögl and Fries medium always resulted in rather sparse growth. By comparison, the medium of Robbins and Schmidt always resulted in abundant growth though producing only 1 to 2 μg/ml of riboflavin. The following is an example of the dry weight of mycelium produced per 100 ml of medium in 5 days in the two media: Kögl and Fries medium—99 mg dry weight; Robbins and Schmidt medium—532 mg dry weight.

Based on the results obtained with the Kögl and Fries medium, attempts were made to combine the riboflavin producing capacity of the one medium with the excellent growth obtained in the second.

TABLE V

RIBOFLAVIN (μG/ML) PRODUCED IN THE ORIGINAL AND MODIFIED KÖGL AND FRIES MEDIA

Original medium		Modified medium	
Organic inoculum	Synthetic inoculum	Organic inoculum	Synthetic inoculum
4	1	52	14

The Robbins and Schmidt medium differs from the Kögl and Fries modified medium, particularly in the absence of Tween 80 and ammonium acetate and in the presence of a number of heavy metals and asparagine.

The ingredients singly and in groups were "criss-crossed" in an attempt to combine the best features of the two formulae. Over several experiments including both inoculum A and "defined" inocula, only the use of ammonium acetate and Tween 80 resulted in any consistent production of fibroflavin in the Robbins and Schmidt medium. The omission of heavy metals in the various combinations of the two media had no effect on ribroflavin yields. The omission or reduction in amount of asparagine also tended to result in better potencies. In general there was more difficulty in reproducing riboflavin yields from experiment to experiment with the media based on the Robbins and Schmidt formula than with that of Kögl and Fries. In all media based on the Robbins and Schmidt formula the amount of growth was consistently good. The final modified medium of Robbins and Schmidt was as follows:

Sucrose	20 gm
KH_2PO_4	0.5 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
$NH_4C_2H_3O_2$	5.0 gm
DL Asparagine	1.0 gm
Boron	0.01 ppm
Molybdenum	0.02 ppm
Iron	0.2 ppm
Zn	0.18 ppm
Cu	0.04 ppm
Mn	0.02 ppm
Biotin	0.4 μg
Thiamin·HCl	0.2 mg
Meso inositol	0.2 gm
Tween 80	1% vol./vol.
Distilled water	to make one liter

DISCUSSION

Riboflavin yields as high as 1760 μg/ml with *A. gossypii* have been reported in the literature (**6**, **8**) using media containing complex substances such as corn steep liquor and peptone. To the author's knowledge no riboflavin production by this fungus has been reported using a defined synthetic medium. In contrast to the results with *A. gossypii*, there are several reports of riboflavin production in synthetic media by the closely related fungus, *Eremothecium ashbyi*. Dulaney and Grutter (**1**) using a medium containing vitamin-free casein, mineral salts, biotin, thiamin and inositol obtained yields of up to 100 μg/ml. Yaw (**10**) reported yields of over 200 μg/ml by supplementing the medium with certain amino acids. In a recent paper, Hickey (**2**) reports the addition of glutamic acid and sodium oleate to a mineral salts-vitamin medium to result in yields of up to 590 μg/ml. He also reports that Tween 80 and lecithin were stimulatory while Tween 40 was not.

In the present work, ammonium acetate and Tween 80 consistently promoted riboflavin production by *A. gossypii* in two different synthetic media. This stimulation was independent of the type of inoculum, that is whether the inoculum was grown in a synthetic or in a complex (corn steep-peptone) medium. The yields with the latter inoculum were always higher but this was apparently not due to a carryover of complex materials from the inoculum since washed inoculum from the organic medium was as effective as unwashed inoculum. Also the addition of corn steep and peptone to the fermentation medium in the amount which could be carried over, did not improve yields when a synthetic inoculum was used.

The reason for stimulatory effect of ammonium acetate is not known. Acetate is known to be incorporated into the riboflavin molecule (**5**). In other fermentations, ammonium acetate performs an important func-

tion in pH control (3). Though no data are available on the pH during the runs, the pH was measured after the completion of the fermentation and no consistent pattern could be found which correlated with the presence of acetate.

A number of other Tweens in preliminary experiments were also found to stimulate riboflavin production. These were Tween 20, 40 and 60 (polyoxyethelene sorbitan monolaurate, monopalmitate, and monostearate respectively). The sorbitan monopalmitate, monostearate and monooleate which are the Span series also were as stimulatory to riboflavin production as were the Tweens. However, the commercial grade fatty acids which make up part of the Tweens and Spans resulted in marked inhibition of growth and riboflavin synthesis. These experiments were run at a pH of 6.8 where the fatty acids would be predominantly in the form of their soaps. The soaps may have been toxic at the levels of fatty acid tested. Lower concentrations might have been stimulatory (2). The Tweens and Spans are all surface active materials and their action in promoting riboflavin synthesis may depend on this property.

SUMMARY

1. Two different synthetic media were found to permit the production of riboflavin by *A. gossypii* if ammonium acetate and Tween 80 were included.

2. Higher riboflavin yields were obtained with the use of inoculum grown on a complex organic medium than with inoculum grown on a synthetic medium, but this was not found to be due to a carryover of nutrients.

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LITERATURE CITED

1. **Dulaney, E. L. and F. H. Grutter.** 1950. The nutritional requirements of *Eremothecium ashbyi* Guill. Mycologia **42**: 717–722.
2. **Hickey, R. J.** 1953. Some nutritional requirements for biosynthesis of riboflavin by *Eremothecium ashbyi*. Jour. Bact. **66**: 27–33.
3. **Jarvis, F. G. and M. J. Johnson.** 1947. The role of the constituents of synthetic media for penicillin production. Jour. Amer. Chem. Soc. **69**: 3010–3017.
4. **Kögl, F. and H. Fries.** 1937. Über den Einflusz von Biotin, Aneurin und Meso-Inosit auf das Wachstum verschiedener Pilzarten. Ztschr. f. physiol. Chem. **249**: 93–110.

5. **Plaut, G. W. E.** 1953. Mechanism of riboflavin synthesis. Fed. Proc. **12**: 254.
6. **Pridham, T. G. and K. B. Raper.** 1950. *Ashbya gossypii*—its significance in nature and in the laboratory. Mycologia **42**: 603–623.
7. —— **and** ——. 1952. Studies on variation and mutation in *Ashbya gossypii*. Mycologia **44**: 452–469.
8. ——, **H. H. Hall and V. G. Pfeifer.** 1950. Production of riboflavin (vitamin B_2) with *Ashbya gossypii*. AIC 271. Northern Regional Research Laboratory.
9. **Robbins, W. J. and Mary B. Schmidt.** 1939. Preliminary experiments on biotin. Bull. Torr. Bot. Club **66**: 139–150.
10. **Yaw, Katherine E.** 1952. Production of riboflavin by *Eremothecium ashbyi* grown in a synthetic medium. Mycologia **44**: 307–317.

THREE SPECIES OF ECCRINALES INHABITING THE HINDGUTS OF MILLIPEDS, WITH COMMENTS ON THE ECCRINIDS AS A GROUP

ROBERT W. LICHTWARDT [1]

(WITH 29 FIGURES)

Although reported in the literature over a century ago by the American naturalist Leidy (**1849**), who was trained as a medical doctor, the Eccrinales seem to have been generally overlooked by mycologists in the United States until attention was called to them by the inclusion of a brief account of the group in relatively recent mycological textbooks by Wolf and Wolf (**1947**) and by Bessey (**1950**). Work already published on the group has been carried out for the most part by biologists in Europe, especially in France. Some very interesting observations have been reported that should stimulate mycologists to look for and to study carefully representatives of this group. Its affinities to other fungi are not clear, although they are included generally among the Phycomycetes, because of the incompleteness of material for observations, contributing to a lack of an understanding of complete life cycles.

Two recent publications of a monographic nature, one by Duboscq, Léger, and Tuzet (**1948**), the other by Manier (**1950**), have summarized most of the accumulated knowledge on the Eccrinales. These commendable publications serve to illustrate the incompleteness of the life cycles of many of the described species and genera, a situation brought about by the difficulties of making observations on the developmental stages, which lead to a confusing and inadequate classification and debatable phylogenetic interpretations regarding these and related fungi. The

[1] The writer is greatly indebted to Dr. Leland Shanor for suggesting that a study of this group of fungi be undertaken, and for his supervision and assistance in the course of the work pursued at the University of Illinois. The first material was collected and preliminary observations made at the University of Michigan Biological Station, Douglas Lake, Michigan, during the summer of 1950. A preliminary report was presented at the 18th Annual Meeting of the Mycological Society of America, at Minneapolis, Minnesota, September 12, 1951. The writer wishes to thank Miss Thelma Howell, Executive Director, and The Highlands Biological Station for a grant from the Col. and Mrs. John S. Sewell Research Fund for studies at the Highlands laboratory during the summer of 1952.

more recent shorter contributions on the Eccrinales and related fungi by the two eminent French investigators on this group, Odette Tuzet and Jehanne-Françoise Manier (**1950**, **1951a**, **1951b**, **1952**), published in the Annales des Sciences Naturelles, Zoologie, provide additional observations which have served to clarify some of these problems.

Members of the order Eccrinales are found attached to the digestive tract of Arthropods. Each individual consists of a disk-like holdfast from which arises a relatively long, thin, unbranched, coenocytic hypha (FIGS. 1, 2, 3). Reproduction is accomplished by means of asexual spores of various types. These spores typically are formed so that they occupy the distal end of the attached hypha, but in some cases the entire hypha may become converted into a chain of spores. Apparently all hyphae are potentially capable of producing reproductive units.

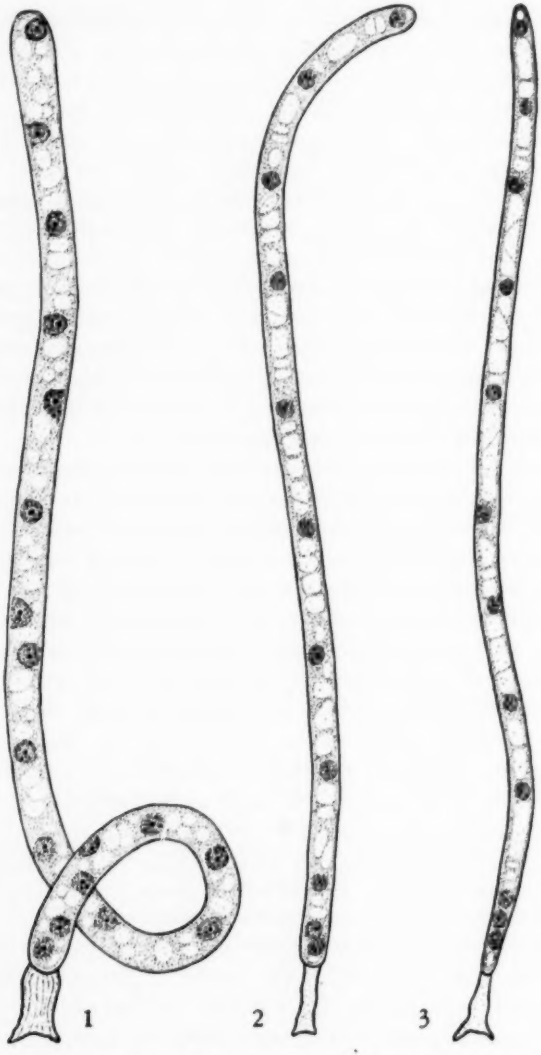
The characteristics of the vegetative hyphae are of some diagnostic value. The filament may be virtually straight, slightly incurved, or may be spiraled near the base. Spiraling may occur to such an extreme that a hypha appears like a small coiled spring. The filaments may reach a centimeter or slightly more in length, but usually are much shorter. The diameter also varies with the species and, although frequently less than 20 μ, may be as much as 90 μ in extreme cases.

The hyphae are generally hyaline, especially when young, or they may be yellowish to brown, depending upon the species or on age and physiological condition. The cytoplasm usually contains conspicuous vacuoles so that in some species it appears reticulate. Some hyphae appear very granular, occasionally to such an extent that the cytoplasmic granules, in stained material, obscure nuclei and other intracellular detail. Oil globules are very common, and often abundant, in the cytoplasm. The nuclei of the vegetative hyphae may be scattered, or are found in a single row more or less equidistant from each other, the latter condition usually preceding spore formation.

According to reports of previous investigators, the walls of hyphae contain cellulose, and its presence has been demonstrated in the course of this study by the use of a solution of potassium iodide followed by application of 70% H_2SO_4. The wall is firm but slightly elastic, and is smooth. Aggregates of filamentous bacteria are frequently found attached to the hyphal wall and around the holdfast.

Duboscq, Léger, and Tuzet (**1948**) and Manier (**1950**) report phenomena in the life cycles of certain species of Eccrinales which these authors interpret as sexuality, but it is apparent that additional and more complete observations will be necessary before the general nature of sexuality in the group becomes clear.

Somewhat similar in structure to the Eccrinales, and also found attached to Arthropods, is a group of fungi classified as the Amoebidiales. These apparently differ from the Eccrinales principally in their production of amoeboid cells, but they may possess also non-amoeboid repro-



FIGS. 1–3. Young, short vegetative hyphae. 1. *Enterobryus elegans*, × 400. 2. *Enterobryus curyuri*, × 400. 3. *Enterobryus apheloriae*, × 400.

ductive structures. Members of the Amoebidiales have simple holdfast disks, their hyphae are generally shorter and broader than those of the Eccrinales, and they are to be found attached to the exoskeleton of the host rather than to the lining of the gut.

Because of their small size and rather unusual habitat, fungi belonging to the Eccrinales are infrequently collected and studied. They may be found without difficulty in Diplopods (millipeds), but also a few species have been reported in terrestrial or aquatic Coleoptera and fresh water or marine Crustacea. They are found frequently also attached to the cuticula of nematodes inhabiting the guts of millipeds. Apparently the Eccrinales are not parasites, but rather live within the host as commensals.

The distribution of the Eccrinales and the closely related Amoebidiales is probably world-wide. Representatives have been found in North, Central, and South America, in Europe, in Africa, and in Indochina. Undoubtedly future investigations will reveal many new localities and interesting forms of these fungi.

The present paper presents additional observations on *Enterobryus elegans* Leidy, the species first described by Leidy in 1849 and 1853 from the milliped *Spirobolus americanus* (*Julus marginatus*), and reports the results of a study of two previously undescribed species that occur in other millipeds common in parts of Illinois (Lichtwardt, **1951**).

MATERIALS AND METHODS

The best material for study of the Eccrinales has been obtained from live hosts freshly dissected. Preserved host specimens have been used, though the information obtained from a study of the hyphae within them usually has decided limitations. For a supply of fresh material for study, millipeds have been kept alive satisfactorily in the laboratory among moldy leaves in suitable containers. This is advantageous, because the available population of most species of milliped in nature varies considerably throughout the year.

Brief mention should be made of some landmarks of the hindgut of millipeds to keep in mind while making a preparation for study. Anteriad in the region of the hindgut is a pronounced sphincter, followed by a ring of fatty substance and two Malpighian tubules, after which the gut again enlarges in diameter. The portion of the gut where the Malpighian tubules enter and where the fatty substance is found marks the borderline of the hindgut and midgut. Eccrinales are always found in the hindgut which is lined with a chitinous layer. The midgut is lined with a peritrophic membrane to which may be attached gregarines,

bacteria, and sometimes nematodes. During my examination of a large number of preparations I have never found any Eccrinids anterior to the hindgut.

During this study, experience has demonstrated that a satisfactory dissection of a milliped to provide good living material for study can be accomplished fairly easily as follows: first, the anal segment and head of a live specimen are cut off. The posterior portion of the hindgut is grasped with a pair of forceps and the gut removed from the body by pulling gently. Next, starting posteriorly and working forward, the hindgut is cut open with a pair of very fine-pointed scissors. Care must be exercised to avoid deep penetration of the scissors into the gut. For the dissection of very small guts it has been found helpful to use a fine insect pin, mounted in an appropriate holder, which has been flattened and sharpened to a cutting edge. After the gut has been cut open, it is flooded with water to spread the hyphae. Under these conditions they radiate from the gut and, if present in large numbers, are visible even to the unaided eye, but the use of a high-powered dissecting microscope is necessary to detect small individual hyphae.

Two methods have been used in fixing, staining, and mounting the material for microscopic study. The first has been more commonly used and has been found quite satisfactory for most observations, except possibly for the study of certain cytological details. In this method the chitinous lining of the hindgut is carefully stripped from the gut, or at least that portion of it to which fungi are attached, and the transparent lining is mounted on a slide in lactophenol (Amann's medium) containing cotton blue or acid fuchsin. A coverslip then is placed on it and the coverslip is ringed. In some cases it has been found difficult or impossible to pull off neatly the chitinous lining. However, if such guts are placed to one side of a Petri dish half filled with water and about twenty drops of lactophenol are placed in the opposite side and allowed to diffuse through the water, within a few hours the lining usually can be stripped off with ease.

The second method employed involves immediate fixation of the cut-open gut in a fresh solution of Flemming's weak chrom-osmo-acetic acid for five minutes. After a thorough washing in water, the chitinous lining with the attached fungi is removed from the gut and stained with Heidenhain's iron-alum hematoxylin. The writer has found this stain to be more satisfactory than cotton blue or others to demonstrate protoplasmic detail. Counterstains such as safranin, eosin, or erythrosin may be used, but as a rule these appear to obscure detail rather than enhance it. The chitinous lining may be removed more easily prior to

staining if the gut has been treated, subsequent to fixation and washing, with lactophenol as described above.

Following staining with Heidenhain's hematoxylin, the attached fungi are placed in 25% glycerin and the glycerin is allowed to concentrate by evaporation. The material is mounted in glycerin and the coverslip ringed.

Attempts to mount in balsam or a similar medium have been less successful, because the very delicate hyphae are susceptible to collapse, even if a gradual alcohol and hygrobutol series is used to dehydrate. In addition, the material becomes very brittle in this mounting medium.

Hyphae still attached to the transparent lining of the gut are mounted with greater ease than when removed from it. An important advantage is that many stages of development within a single host can be studied in a single preparation.

Attempts have been made to obtain these fungi in culture, but, although numerous techniques and media have been employed, I have not yet met with success.

OBSERVATIONS

Spore Types Found in the Eccrinales: One of the striking characteristics of members of the Eccrinales is the variation in spore types which may be formed. In some species only a few kinds of spores have been seen, while in others more than half a dozen different types of spores have been reported.

The variability in spore types and the difficulty in adequately interpreting their method of formation and germination has led to a confusing and inaccurate terminology. Current usage of terms as these are employed for designating spore types in the Eccrinales have been those suggested principally by Léger and Duboscq. These investigators, even in their last publication (Duboscq, Léger, & Tuzet, **1948**), are not consistent in the application of their own terminology, thus leading to some confusion. Manier (**1950**) follows the same basic scheme of spore designation, but is somewhat more consistent in her application. Even so, her usage of the terms macroconidia and microconidia is frequently contrary to generally accepted mycological practice. In some cases these terms as used may be applicable, but in other cases they refer either to what are obviously not conidia, or to spores whose method of development is not known and which consequently cannot be conclusively so designated.

In view of the present status of terminology on spore formation and function as applied to the Eccrinales, it seems to me preferable to desig-

nate the spore types which have been found in the three species of *Enterobryus* described in this paper in the following manner:

SPORE TYPE A (FIG. 4A). Spores of this type are borne singly within sporangia which are initiated by regular formation of crosswalls at evenly spaced intervals from the apex of the hypha toward the base. Each escapes from the sporangium by way of a lateral hole or break in the sporangial wall. At discharge these spores have a delicate wall and usually contain four or eight nuclei. This spore is perhaps the most universally found in the Eccrinales and apparently serves as a common

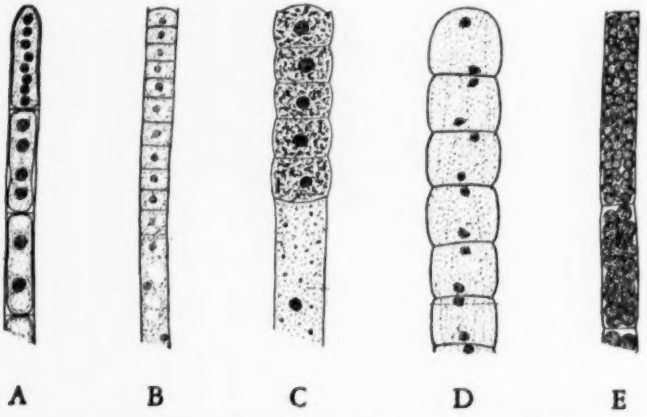


FIG. 4. Spore types found in three species of *Enterobryus*, all × 400. A. Sporangiospores of *E. curyuri* (also found in *E. elegans* and *E. apheloriae*). B. Endoconidia of *E. curyuri*. C. Uninucleate spores of *E. curyuri*. D. Binucleate spores of *E. apheloriae*. E. Multinucleate spores of *E. curyuri* (also found in *E. apheloriae*).

means of rapid reproduction within the host. In the literature, this spore type is referred to commonly either as a macroconidium or, sometimes, as a macrospore. All three species of *Enterobryus* considered in this paper produce spores of this type.

SPORE TYPE B (FIG. 4B). Spores of this type may be regarded as endoconidia. They are uninucleate and are formed by successive cleavage from the hyphal protoplasm. They escape through the opened end of the hypha. Perhaps they assume a spherical shape after emerging, for occasional hyphae have been found in which one or two terminal conidia were rounded. These spores have been referred to frequently by other workers as microconidia or microspores. I have found them in *Enterobryus curyuri*, described below, and they have been reported as common in many other species of Eccrinales.

SPORE TYPE C (FIG. 4C). Spores of this type, although somewhat superficially resembling the spores of type B, are evidently formed by the successive cleavage of the filament so that the original hyphal wall forms the lateral wall of the spore. Spores of type C may be properly designated as oidia. They are found only at the terminal end of the hyphae. They bear a resemblance to spores which have been reported for other species of Eccrinales, such as *Enterobryus rectus* Lég. & Dub. and *Trichella helocharei* Poisson. In the course of the present investigation, spore type C has been seen only in *Enterobryus euryuri*.

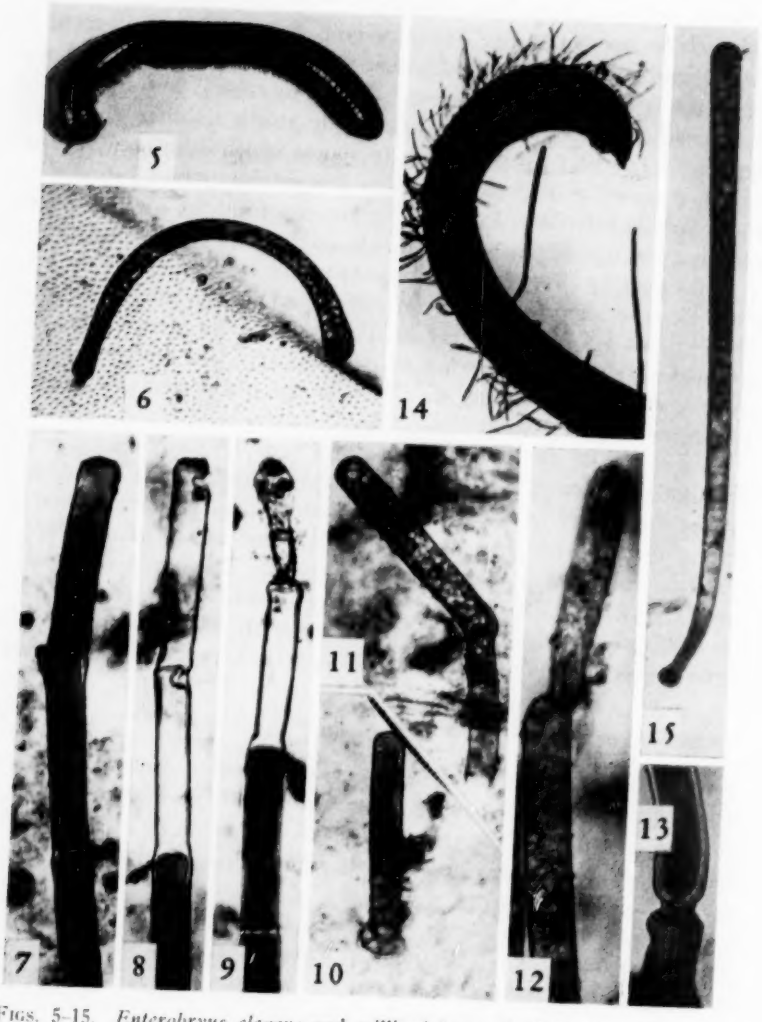
SPORE TYPE D (FIG. 4D). Filaments producing this spore type eventually become almost entirely converted into these reproductive units. These spores are large, roughly isodiametric, and the more mature ones are binucleate. The writer has found them only in *Enterobryus apheloriae*, described below, and they have not been reported previously in the literature on Eccrinids.

SPORE TYPE E (FIG. 4E). Spores of type E are characterized by containing dozens of nuclei tightly packed within the cell. They have the same diameter as the hyphae from which they are formed and are several times longer than they are wide. One to six of these spores constitute the usual number found on a hypha. This represents another spore type which does not appear to have been previously described for the Eccrinales, though they are not uncommonly found in individuals of *Enterobryus euryuri* and *Enterobryus apheloriae*, especially the former.

ENTEROBRYUS ELEGANS Leidy

Enterobryus elegans was first described by Leidy in 1849, and in 1853 was illustrated and further characterized. While my own observations confirm most of Leidy's, they also make it possible to supplement the information he has provided regarding this species and to emend certain of Leidy's statements.

The host of *Enterobryus elegans* is the common eastern milliped *Spirobolus americanus* (Beauvois) (FIG. 5), formerly known as *Julus marginatus* or *Spirobolus marginatus* (Hoffman, 1951). The material studied was obtained principally from the vicinity of Douglas Lake, Michigan, and from Champaign County, Illinois, though a few individuals from other parts of the country, especially from the region around Highlands, North Carolina, have been examined. The northern Michigan millipeds obtained usually have been larger in size and have harboured a larger number of nematodes within them than was found within the millipeds of Champaign County. The individuals of *Enterobryus*



FIGS. 5–15. *Enterobryus elegans* and milliped host. 5. *Spirobolus americanus* (Beauvois), host of *E. elegans*, × ¾. 6. Young hypha attached to chitinous lining of hindgut, × 250. 7–12. Stages in emergence and germination of spore type A, × 480. 13. Holdfast, × 480. 14. Part of a nematode bearing two kinds of hyphae, × 65. 15. Sporangiospore found attached to nematodes during molting of *S. americanus*, × 250.

elegans from the different collections show a slight variation from one host locality to another, but they are so similar that differences are interpreted as within the range of specific variations.

Young vegetative filaments of *Enterobryus elegans* (FIG. 1) are usually highly vacuolate. Some hyphae possess very granular cytoplasm and such filaments are often slightly wider than the non-granular hyphae. The basal portion frequently exhibits a pronounced curve or spiral. The free end of a vegetative hypha may appear slightly clavate (FIG. 6), though such a condition is not universal in the species. The holdfast in most cases is broadened at the base into a disk which is attached to the host. The holdfast stalk may be as long as 40 μ, though it is usually shorter, and it sometimes shows longitudinal striations (FIG. 13). Hyphae are attached most abundantly to the anterior portion of the hindgut.

The hyphae are almost always under 4.5 mm in length, and usually have a diameter of 19–23 μ. Sporulating hyphae have been found, however, that may be either much wider or much narrower. Usually the cell wall is thin, but some specimens from the Douglas Lake region have walls up to 2.8 μ thick. This greater thickness in cell wall often characterizes hyphae of greater diameter than usual.

Nuclei are demonstrated to be numerous and prominent when hyphae are stained. They usually measure 4–5.6 μ. Leidy neither makes any reference to the nuclei nor shows them in his numerous drawings, possibly because the majority of his observations were made on unstained material.

Only spore type A, the sporangiospore, has been found in *Enterobryus elegans*. These seem to follow a simple pattern of development. Prior to formation of the sporangia, the nuclei in a hypha are seen to be arranged linearly and equidistant. Behind the terminal nucleus a crosswall is formed to delimit a cell, the first sporangium. The nucleus in this cell undergoes a division to give rise to two daughter nuclei, each of which undergoes two more divisions to produce eventually a cell with eight nuclei. In the meantime a second portion of the protoplasm within the hypha containing the original penultimate nucleus may have been cut off by a crosswall to produce a second cell, the second sporangium. This formation of uninucleate cells, which later become typically 8-nucleate, may continue progressively backward until virtually the entire hypha has become converted to sporangia, though at any one time during this development only a few sporangia may remain on the filament. Crosswall formation may take place relatively rapidly so that the second cell is formed when the terminal spore has but two nuclei, or crosswalls

may be relatively slow in forming so that the terminal cell may contain eight nuclei before the penultimate cell is cut off.

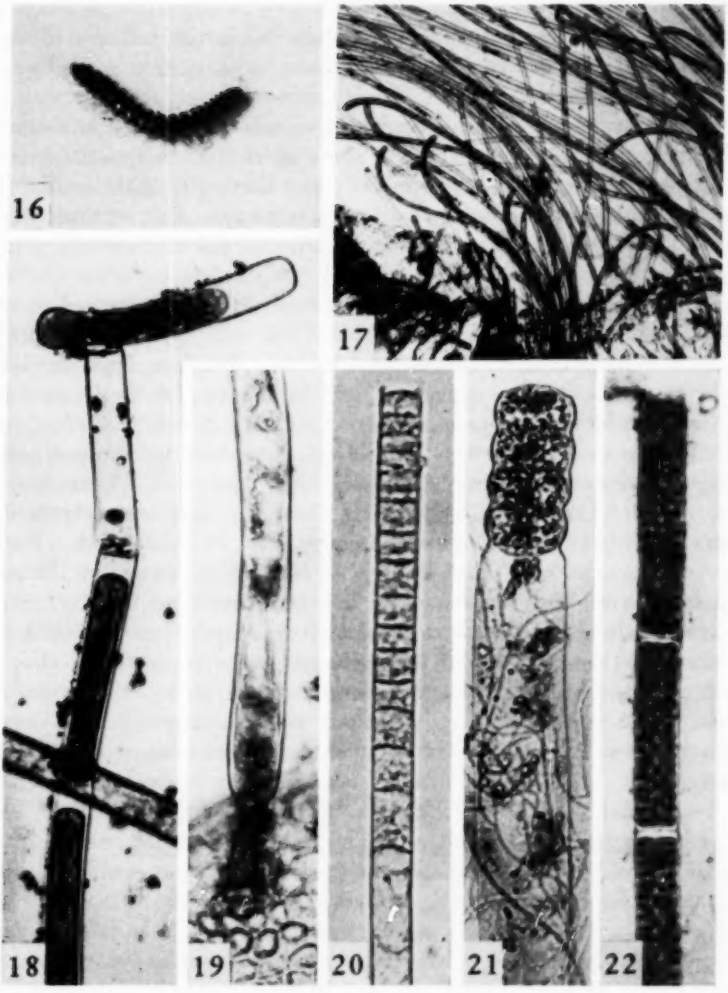
The division of the nuclei within each sporangium appears to occur simultaneously (see Fig. 27), since one, two, four, or eight nuclei per cell are the numbers most often encountered. However, spores bearing three, five, or seven nuclei are fairly frequent, indicating that the divisions are not entirely synchronized within the cell.

When the spore is ready to emerge from the sporangium it is usually in the 8-nucleate condition, and with one end of the spore appearing slightly bulbous. Spores may emerge either from the distal or proximal regions of a sporangium, most frequently from the distal end, but seemingly consistently at one or the other for all sporangia formed from any hypha. The first sign of emergence is a dissolving of the lateral wall at the end of the sporangium (FIG. 7) where the bulbous end of the spore is located. The spore then emerges through this opening, and if it is not the terminal spore, forces the hypha back to permit its escape (see FIG. 18). After release of the spore (FIG. 8), the old sporangial wall either decomposes *in situ* or breaks off and decomposes. Bacteria appear to play an important role in this decomposition, for the empty sporangia are usually largely covered with bacteria (FIG. 9).

The newly escaped spore becomes attached to the chitinous layer of the hindgut (FIG. 10) by the end which first emerged from the sporangium. In rare cases the unemerged spore already shows the rudiment of a holdfast, but in most cases no such structure is discernible until after attachment to the gut. After fixation to the substratum, the process of spore germination begins. This consists of a growth of the hypha between the point of attachment and the spore case (FIG. 11). The elongating hypha, therefore, pushes the spore case away from the holdfast. As the hypha elongates, the spore case remains attached to the end of the filament, and is usually slightly reflexed.

In the early stages of hyphal elongation the nuclei may divide infrequently, but more often nuclear multiplication keeps pace with elongation of the filament, with a few of the nuclei remaining within the spore body. After a period of growth of the hypha, a wall begins to form at the region separating the main part of the hypha from the spore (FIG. 12). At this stage there may be little protoplasm remaining within the spore, and by the time the crosswall is complete the spore case appears empty except for a few scattered granules.

There is apparently no correlation between the disappearance of the spore case and the length of the hypha, for in some cases very short hyphae may have already lost the spore case while in other filaments



FIGS. 16–22. *Enterobryus curyuri* and milliped host. 16. *Euryurus erythropygus* (Brandt), host of *E. curyuri*, × 1. 17. Small section of gut lining with hyphae attached, × 66. 18. Spore type A in process of emerging from sporangium, × 480. 19. Holdfast, × 480. 20. Sore type B, × 480. 21. Spore type C, × 480. 22. Spore type E, × 480.

there is considerable elongation before the spore case finally disintegrates and disappears. Bacteria again probably play an important part in the decomposition of the spore wall, since these microorganisms are always found associated with the decomposing spore case, as they are with the old empty sporangia.

A close examination of a germinating filament in the region where the hypha proper emerges from the spore reveals not only a continuity of the protoplasm of the two parts but also a continuity of the walls. It appears that the expanding hyphal wall is not a new wall, separate from the wall forming the spore case, but rather an extension of the spore wall itself.

Hyphae of *Enterobryus elegans* are frequently found attached to the cuticula of the nematodes which inhabit the milliped gut (FIG. 14). Specimens of *Spirobolus* from Douglas Lake, Michigan, have an especially abundant fauna of nematodes, and hyphae may be found attached to any of the three species of nematode normally found in the hindgut. The hyphae thus attached may be identical to those on the gut itself, and produce spores of type A like those already described. These spores are frequently shorter (32–48 μ long), however, and escape from the sporangium from the proximal end rather than the distal end. These shorter spores are also found on hyphae attached to the wall of the gut, but are less frequently encountered than the longer type.

The attachment of hyphae to the nematodes may be merely by chance. It should be noted, however, that millipeds examined in the process of molting provide some evidence which suggests that the relationship between the nematode and the fungus is more complex than casual observation would indicate. In the process of maturing, millipeds molt many times. With each molt they shed the lining of the hindgut which is ectodermal in origin, along with the exoskeleton. Therefore, any hyphae attached to the hindgut lining are shed with the molt. Ordinarily only a relatively small percentage of nematodes in a milliped bear hyphae of *Enterobryus*, but in millipeds about to molt, virtually all the nematodes, at least the more mature ones, were seen to be infested with Eccrinids. The number of hyphae that may be found on different nematodes varies usually from a few to over fifty, but in extreme cases hyphae were too abundant to be counted. Furthermore, not only was the actual number of hyphae attached to nematodes just before a molt greater, but the filaments on them seemed to be somewhat different.

Apparently just before the host molts the Eccrinids are stimulated to produce a modification of spore type A which is somewhat different from the characteristic type (FIG. 15). These spores are usually 13–16 μ

wide, and may exceed 300 μ in length. The base of the spore is generally slightly curved and terminated by an adhesive, bulbous tip which becomes attached to the substratum. It appears that these spores frequently elongate considerably before germination and this may account for their extreme length in some cases.

In addition to hyphae bearing the elongate spore type A, a very large number of hyphae frequently were found attached to the nematodes which are about 6.5–8 μ wide and which may measure up to 100 or 200 μ in length (FIG. 14). These smaller hyphae frequently have transverse or oblique septations which divide the hyphae into binucleate cells. Their holdfasts are small and simple. It has not yet been ascertained whether this is merely a phase in the life cycle of *Enterobryus elegans* or whether they actually belong to a different Eccrinid. It is possible that these small hyphae are directly related to some other small, curved hyphae which may be found either densely or loosely aggregated in the posterior part of the hindgut near the rectum in many specimens of *Spirobolus*. Leidy illustrated some hyphae of this kind from the gut (Leidy, **1853**, plate II, figs. 2 & 3), and considered them to be simply young stages of *Enterobryus elegans*. They seem quite distinct, however, from the larger, more conspicuous hyphae generally found, even though they may be proved, at some later date, to belong to the life cycle of the same species.

The stage on the nematodes may function to help retain the fungus within the gut during molting when the lining of the gut is shed, for the majority of the nematodes remain within the gut. Since this species of milliped usually eats its own molt, there is the possibility that any spores clinging to the molt may also serve to quickly recontaminate the host, but as yet no evidence has been gathered to support this supposition.

Enterobryus euryuri sp. nov.[2, 3]

Hyphis sterilibus cum apicibus saepe curvis, basi raro spirali. Hyphis saepius minus quam 4 mm longis; 10–13μ diam., nonnumquam cum hyphis fertilibus usque

[2] The generic name originally used by Leidy was *Enterobrus*, derived from the two Greek words ἔντερον and βρίον. This name was corrected to *Enterobryus* by Leidy in 1850 without an accompanying explanation. Since the name *Enterobryus* is orthographically more correct than the original spelling of the name, *Enterobryus* is considered valid under Article 82 of the International Code of Botanical Nomenclature adopted in 1950. The desirability of using *Enterobryon* in preference to either *Enterobrus* or *Enterobryus* was suggested by Tuzet (Duboscq, Léger, & Tuzet, **1948**, p. 32), but such a change cannot be regarded as an orthographic correction since Leidy clearly intended the generic name to be in the masculine gender.

[3] The writer acknowledges with appreciation the assistance of Dr. F. D. Lazenby, Department of Classics, University of Illinois, in the preparation of the Latin diagnoses.

ad 90 μ prope basim. Cytoplasmate hyalino, reticulatissimo, aliquando granosissimo. Retinaculo angusto, usque ad 75 μ vel plus longo, cum vel sine orbi basali. Sporangiosporiis (typo A) circa 50–100 × 10–13 μ, ex foramine laterali ad terminum sporangii proximum evenientibus. Endoconidiis (typo B) 10–13 μ diam., ex termino hypharum evenientibus. Sporis uninucleatis (typo C) circa 11 μ long. et 22 μ lat., nonnumquam in hyphis latissimis. Sporis multinucleatis (typo E) circa 11 μ lat. et 35–60 μ long.

Hyphis intestino posteriori *Euryuri erythropygi* (Brandt) adjunctis.

Attached to lining of hindgut of the milliped *Euryurus erythropygus* (Brandt). Vegetative hyphae with tips frequently curved, base seldom spiraled. Length of hyphae usually under 4 mm; diameter typically 10–13 μ, occasional sporulating hyphae up to 90 μ near base. Cytoplasm hyaline and highly vacuolate, occasionally very granular. Holdfast narrow, up to 75 μ or more in length, with or without basal disk. Sporangiospores (type A) about 50–100 × 10–13 μ, emerging from a lateral pore at the proximal end of the sporangium. Endoconidia (type B) 10–13 μ in diameter, emerging from the end of the hypha. Uninucleate oidia (type C) about 11 μ in length by 22 μ in diameter, occasionally borne on very broad hyphae. Multinucleate spores (type E) about 11 μ in diameter by 35–60 μ in length.

Specimens of the host, *Euryurus erythropygus*[4] (FIG. 16), examined in the course of this study have been collected principally in Champaign County, Illinois. The hyphae of *Enterobryus euryuri* have been found attached to the hindgut of the milliped, especially near the anterior portion where the gut is considerably narrowed by the sphincter muscle. Filaments, many of which were covered with filamentous bacteria, have been found also as far back as the rectum. Well infested hosts bear great numbers of hyphae (FIG. 17), though some were found with few filaments, and in rare cases, none. Nematodes also were found in the gut, sometimes in large numbers, but hyphae of this species have not yet been found attached to any of these nematodes.

The holdfast (FIG. 19) is quite variable in size and shape. In some cases the basal disk is large in proportion to the length of the holdfast stalk, while on other filaments the stalk of the holdfast may be much elongated (75 μ) and relatively narrow (4 μ) with no conspicuous basal disk. A typical filament has a holdfast measuring 15–25 μ long by 6.5 μ wide, with the base measuring 8.5 μ in diameter. The holdfast stalk is generally uneven in diameter. If the stalk is long, it is frequently bent. Occasionally delicate longitudinal striations may be observed.

Live vegetative filaments (FIGS. 2, 17) are usually hyaline and commonly with curved tips. Some filaments may possess large amounts of

[4] The writer is indebted to Dr. R. V. Chamberlin, University of Utah, for identifying both this milliped and *Apheloria iowa*.

granular material which is confined either to the distal end or occupies completely the length of the hypha. If granular material is present in large quantities, it usually obscures the nuclei and all other details in stained preparations.

Four different types of spores have been observed in this species. Sporangiospores of type A (FIG. 18) have been most frequently seen. The development and germination of this spore in *Enterobryus euryuri* seems to correspond to similar stages for the same spore type in *Enterobryus elegans* already described. The variation in size for this spore type is great. While they usually range within 10–13 μ wide by 50–100 μ long, they may vary in width from 6–16 μ, and in length from 32–100 μ. Usually the larger spores are granular in nature and have larger nuclei than the more common smaller spores.

Spore type B (FIG. 20) has been somewhat less commonly encountered than type A. However, some hosts were found in which the hyphae bear this type of spore more frequently than others. These spores, endoconidia, were found either singly or in a series of as many as a dozen or more. They are formed by the successive division of the protoplast to produce uninucleate cells. The fate of these spores after they leave the hypha has not been determined. A thorough search of the intestinal lining and milliped feces has revealed no sign of the spores of type B, or hyphae which may have originated from such spores.

The width of the endoconidia appears to be fairly constant, usually ranging within 10–13 μ. The length, however, is more variable, ranging from 5–15 μ. The width is determined by the diameter of the hypha, while the length may be dependent upon the rapidity of spore delimitation and the pressures which are exerted upon the cells from below.

Hyphae forming spores of type B frequently measure up to 2 or 3 mm in length. The base of the filament, as a rule measuring between 16 and 21 μ, often tends to be slightly wider than the distal end. The holdfast is usually 23–35 μ long by 12 μ wide. The cytoplasm of many of the hyphae observed producing endoconidia was very granular, especially the cytoplasm of the spores themselves.

Hyphae bearing spore type C (FIG. 21) are of interest because some of these hyphae have the greatest diameter of any filaments reported in the Eccrinales. The diameter is variable, and while many filaments were found measuring only 23–30 μ, extremely broad filaments have been observed which measured about 90 μ, certainly an unusually large diameter for Eccrinid hyphae. Filaments have not been observed which exceeded 4 mm in length.

This spore is structurally simple and measures commonly about 11 $\times$ 22 μ, as a rule being about twice as broad as long. It is uninucleate and

the cytoplasm almost always contains dark-staining granules. Formation of these spores appears to be of the pattern characteristic of oidia. They have never been observed in numbers greater than a dozen on one hypha. No spores of this type have been seen detached from hyphae and nothing is known of their fate or function in the life cycle.

The fourth type of spore produced by *Enterobryus euryuri* is type E (FIG. 22). Previous to formation of spores of this type, hyphae contain a much larger number of nuclei than is found ordinarily in those that are strictly vegetative. These nuclei aggregate at the tip of the filament and then a crosswall is formed, thus delimiting a multinucleate cell. Subsequent crosswalls are formed to produce similar cells, until a sequence of perhaps half a dozen spores results. The fate of these unusual spores has not been determined.

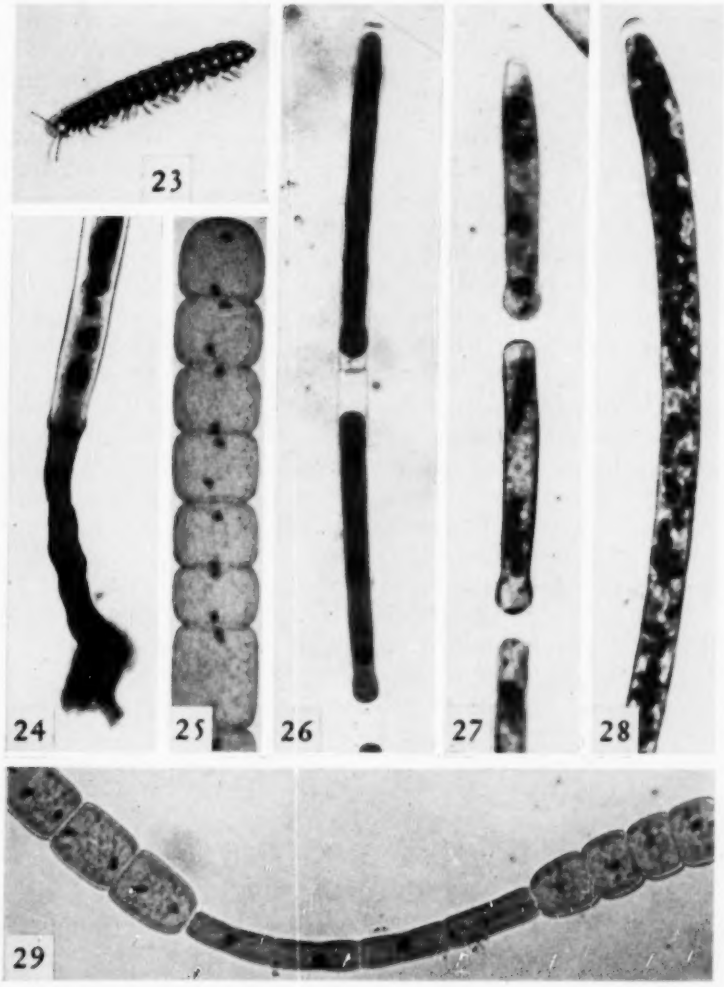
Enterobryus apheloriae sp. nov.

Hyphis sterilibus aliquantum fusiformibus, apice hebeti, basi ad retinaculum paulatim decrescente. Hyphis usque ad 10 mm vel aliquantulo plus longis, sed saepius brevioribus; plerumque 8–12 μ diam. Cytoplasmate hyalino et reticulatissimo. Retinaculo saepius cum orbi vel incremento basali. Sporangiosporis (typo A) saepe longis et angustis, circa 68–265 × 8–12 μ, ex foramine laterali ad terminum sporangii proximum evenientibus. Binucleatis sporis (typo D) circa 40 × 40 μ. Sporis multinucleatis (typo E) nonnumquam praesentibus, circa 60 μ long. et 11 μ lat. Hyphis intestino posteriori *Apheloriae iowa* Chamberlin adjunctis.

Attached to lining of hindgut of *Apheloria iowa* Chamberlin. Vegetative hyphae somewhat fusiform in shape, the tip bluntly pointed, the base tapering rather gradually to the holdfast. Hyphae up to 10 mm long, or slightly more, usually shorter; diameter usually 8–12 μ. Cytoplasm hyaline, highly vacuolate. Holdfast usually with a basal disk or enlargement. Sporangiospores (type A) often long and narrow, about 68–265 × 8–12 μ, emerging from a lateral pore or tear at the proximal end of the sporangium. Binucleate spores (type D) measuring about 40 × 40 μ. Multinucleate spores (type E) occasionally present, measuring approximately 60 × 11 μ.

Material studied has been obtained from *Apheloria iowa* collected mainly in Champaign County, Illinois. Hyphae of *Enterobryus apheloriae*, in varying degrees of abundance, were found attached to the hindgut of the milliped, especially near the anterior part. Sometimes hyphae were not only very numerous but also quite long, some measuring slightly over 1 cm in length. Nematodes may or may not be found inside the gut but no hyphae have been seen attached to the nematodes.

The predominant method of reproduction observed in this species is by spore type A (FIGS. 26, 27). Their method of formation and germination in this species is very similar to that already described for this



FIGS. 23–29. *Enterobryus apheloriae* and milliped host. 23. *Apheloria iowa* Chamberlin, host of *E. apheloriae*, × ¾. 24. Holdfast, × 480. 25. Spore type D, × 300. 26. Spore type A, × 480. 27. Synchronous division of nuclei in spore type A, × 480. 28. Hypha with aggregated nuclei at apex prior to formation of spore type E, × 480. 29. Part of a hypha producing spores of type D, containing several unswollen, uninucleate cells, × 300.

spore type of *Enterobryus elegans*. The general shape of the sporangiospores, as compared with those observed in the other two species, is longer and narrower, and they are quite variable in dimensions. While the length may range from 68–265 μ, it is more commonly between 70 and 100 μ.

A second spore type produced by *Enterobryus apheloriae* is that designated as type D (FIG. 25). Only three millipeds have yielded hyphae possessing such spores, though each individual in which they were found contained a relatively large number. Spore chains measured 60–160 μ in length. In all cases the entire hypha, except for the two or three basal cells, had been converted into these spores. Terminal spores were always binucleate. Although the exact sequence of events in their formation is not known, it seems likely that such hyphae initially become divided into cells which first enlarge considerably in diameter, then, through subsequent divisions of the nuclei and formation of cell walls, these are transformed into the large binucleate spores of type D. Most chains of spores show evidence of intercalary division. In a couple of instances, within chains of maturing spores were found a few uninucleate, narrow cells (FIG. 29) having the approximate diameter of a vegetative hypha. These are interpreted to be cells which have not yet enlarged as have their neighboring cells.

The third kind of spore, type E, produced by *Enterobryus apheloriae* was found in less abundance in this species than in *Enterobryus euryuri*. These spores were almost indistinguishable in the two species, but spores of type E of *Enterobryus apheloriae* occasionally attain a slightly greater length. A hypha which had started to form such spores by the accumulation of nuclei at the apex of the filament prior to formation of the first cross-wall is shown in FIG. 28.

DISCUSSION

In the literature on the Eccrinales and Amoebidiales (the Eccrinids) frequent reference is made to their parasitic nature. Some investigators, i.e., Léger and Duboscq (see Duboscq, Léger, & Tuzet, **1948**), and Poisson (**1929**, **1931**), seem to refer consistently to all Eccrinids as parasites, while others, i.e., Lichtenstein (**1917a**, **1917b**), and Mainier (**1950**), have indicated that at least the externally attached members of the Amoebidiales are commensals. More recently Tuzet and Manier (**1952**) consider even the internal Eccrinids of the genus *Enterobryus* as commensals, and they appear to be so to me.

Seemingly supporting the suggestion that the Eccrinids are commensals is the lack of rhizoids or similar structures extending beyond the holdfast,

and the nature of the holdfast itself which is solid and often of considerable length and not provided with a cytoplasmic core through which nutritive substances from the host might readily diffuse. At the point of attachment of the Eccrinid to the host some physical disturbance of the lining, due to the strong adhesive properties of the holdfast, may be seen, but no evidence has been observed indicating lysis at or beneath the point of attachment. These same observations also hold true in cases where the fungus is attached to nematodes.

For the Eccrinales found attached to the lining of the gut of Arthropods, it appears quite likely that direct absorption of nutritive materials through the hyphal walls occurs. For the Amoebidiales, whose members in some instances may be attached virtually anywhere to the exoskeleton of the host, the source of nutritional substance is not so obvious. However, some simple but interesting experiments performed by Chatton (**1906**) on the effect of environmental conditions on the ectophyte *Amoebidium parasiticum* Cienk. suggest absorption of substances from the surrounding water. He has shown that an insufficient amount of organic material in the water containing infested hosts has a dilatory effect upon the growth of the fungus. It seems reasonable, then, to regard members of both orders of Eccrinids as commensals.

A compilation of data on host specificity indicates that, generally, one species of Eccrinid is confined to one species of host, though such limited specificity is not true in all cases. *Eccrina flexilis* Lég. & Dub., for example, has been reported on six different species of Diplopods of the genus *Glomeris*, and the ectophyte *Amoebidium parasiticum*, on a wide range of Arthropods. Until a much greater number and variety of hosts are searched, and until some of the species which have been named on the basis of limited material are re-examined, we cannot properly arrive at any clear conception of the degree of host specificity in the Eccrinids.

The classification of the Eccrinids rests principally on the proposals of Léger and Duboscq, developed during the course of their investigations. Duboscq, Léger, and Tuzet (**1948**) have proposed the establishment of a separate group, designated as the Trichomycetes, composed of two "classes," the Eccrinids (Amoebidiales and Eccrinales), and the Harpellids (Harpellales and Genistellales). Manier (**1950**), in a subsequent paper, follows the same basic scheme, but has made some rearrangements and amplifications in it. That this system of classification is still unstable is exemplified by further changes made in a recent paper by Tuzet and Manier (**1951**). To me the Trichomycetes, as they now stand, include genera of questionably close affinities to fungi properly recognized as the Eccrinales.

Certain taxa in the Eccrinales might well be subject to reinvestigation. To cite but one example, between 1849 and 1853 Leidy described, diagnosed, and illustrated two genera, *Enterobryus* and *Eccrina*, which he separated solely on the basis of the number of terminal cells (sporangia) produced by individual hyphae. *Enterobryus* reportedly produced one, two, or rarely three of such cells, while *Eccrina* produced a succession of numerous cells. On the basis of the *Enterobryus* material which I have obtained, this distinction between the two genera does not seem to me to be valid. For, while it is true that usually only few sporangia may be present in a hypha at any one time, up to a dozen or more are not infrequently found in *Enterobryus elegans*. Apparently the entire hypha becomes converted successively to sporangia, with the formation of the first one at the apex initiating the process. The number of sporangia to be seen at any one stage depends first upon the rate of crosswall formation, and second, upon the rate at which the spores quit the sporangia and the empty sporangia decompose or are torn off by abrasion. Consequently, the number of cells on a hypha is probably not a good generic character, and it remains to be seen whether both *Enterobryus* and *Eccrina*, as established by Leidy, are actually distinct genera, separable on the basis of other criteria.

Phylogenetic relationships within the Eccrinids and their relationships to other groups are at present difficult to establish in view of the lack of complete information on life cycles and the controversial interpretations that have been expressed concerning some critical morphological characters. Much further investigation is needed to elucidate the nature of this rather strange group of fungi.

SUMMARY

Three commensal fungi in the hindguts of millipeds belonging to the genus *Enterobryus* are described, one a long-established species and the other two new. The first species described and assigned to the genus, *Enterobryus elegans* Leidy, has been studied in more detail and an account given of the development of its spores. What appears to be an interesting adaptation for the maintenance of the fungus within the molting milliped, by attachment to nematodes that also inhabit the gut, is reported. Also described are two other fungi, *Enterobryus euryuri* from *Euryurus erythropygus* (Brandt) and *Enterobryus apheloriae* from *Apheloria iowa* Chamberlin, which are considered to be new species. Five different spore types produced by these three species of *Enterobryus* are described. A technique is outlined for studying members of the genus.

The general characteristics and distribution of the order Eccrinales, and the status of its taxonomy, are discussed.

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LITERATURE CITED

Bessey, E. A. 1950. Morphology and taxonomy of fungi. The Blakiston Co., Philadelphia. 791 pp.

Chatton, E. 1906. Sur la biologie, la spécification et la position systématique des *Amoebidium*. Arch. Zool. Exp. et Gén., Ser. 4 (Notes et Revue 2) **5**: 17–31.

Duboscq, O., L. Léger and Odette Tuzet. 1948. Contribution à la connaissance des Eccrinides. Les Trichomycètes. Arch. de Zool. Exp. et Gén. **86**: 29–144.

Hoffman, R. L. 1951. The name of the common Eastern Spirobolid milliped. The Florida Entomologist **34**: 15–16.

Leidy, J. 1849. *Enterobrus*, a new genus of Confervaceae. Proc. Acad. Nat. Sci. Phila. **4**: 225–227.

——. 1850. Descriptions of new Entophyta growing within animals. Proc. Acad. Nat. Sci. Phila. **5**: 35–36.

——. 1853. A flora and fauna within living animals. Smithsonian Contributions to Knowledge **5**: 1–67.

Lichtenstein, J. L. 1917a. Sur un *Amoebidium* à commensalisme interne du rectum des larves d'*Anax imperator* Leach: *Amoebidium fasciculatum* n. sp. Arch. Zool. Exp. et Gén. (Notes et Revue 3) **56**: 49–61.

——. 1917b. Sur un mode nouveau de multiplication des Amoebidiacées. Arch. Zool. Exp. et Gén. (Notes et Revue 4) **56**: 95–99.

Lichtwardt, R. W. 1951. Studies on some species of Eccrinales inhabiting the intestinal tract of millipeds. Unpublished Master of Science Thesis, University of Illinois.

Manier, Jehanne-Françoise. 1950. Recherches sur les Trichomycètes. Ann. Sci. Nat., Bot., Ser. 11. **11**: 53–162.

Poisson, R. 1929. Recherches sur quelques Eccrinides parasites des Crustacés Amphipodes et Isopodes. Arch. Zool. Exp. et Gén. **69**: 179–216.

——. 1931. Recherches sur les Eccrinides. Deuxième contribution. Arch. Zool. Exp. et Gén. **74**: 63–68.

Tuzet, Odette and Jehanne-Françoise Manier. 1950. *Lajassiella aphodii*, n. g. n. sp. Palavascide parasite d'une larve d'*Aphodius* (Coléoptère Scarabacidae). Ann. Sci. Nat., Zool., Ser. 11. **12**: 465–470.

——. 1951a. Sur quelques Eccrinides du Brésil. Ann. Sci. Nat., Zool., Ser. 11. **13**: 145–147.

——. 1951b. Le cycle de l'*Amoebidium parasiticum* Cienk. Revision du genre *Amoebidium*. Ann. Sci. Nat., Zool., Ser. 11. **13**: 351–364.

——. 1952. Trichophytes commensaux de l'intestin postérieur de Diplopodes du Brésil. Quelques considérations sur les Trichophytes déja décrits infestant les Diplopodes. Ann. Sci. Nat., Zool., Ser. 11. **14**: 249–262.

Wolf, F. A. and F. T. Wolf. 1947. The Fungi. Vol. I. John Wiley & Sons, Inc., New York. 438 pp.

NORTH AMERICAN SPECIES OF GEOGLOSSUM AND TRICHOGLOSSUM [1]

E. B. MAINS

(WITH 62 FIGURES)

Durand published his Geoglossaceae of North America in 1908 based mostly on collections from the eastern United States. He later (**1921**) presented some additional species and information. Papers by Sinden and Fitzpatrick (**1930**) and the writer (**1935, 1940**) have added several species for North America. Seaver (**1951**), in his treatment of North American inoperculate cup-fungi, has followed Durand's treatment of the Geoglossaceae, incorporating the additional species. Since Durand's studies a number of papers have been published concerning the family for Europe and Asia which have added considerable information involving new species and reevaluations of genera and species with changes in concepts.

Interest in the Geoglossaceae was first developed by the writer as a graduate student. Since 1930 special attention has been devoted to the family. The active cooperation of Dr. A. H. Smith in collection of the group in his field studies has furnished much material and especially has added collections from the northwestern United States. Collections loaned from the Herbarium of the University of California have also furnished western material. In addition critical collections have been available from other herbaria which have greatly furthered the study. In indicating the herbaria in which the specimens are found the following designations are used: (BPI) Mycological Collections, U. S. Bureau of Plant Industry, (CUP) Herbarium of the Department of Plant Pathology, Cornell University, (FH) Farlow Herbarium, Harvard University, (MICH) Herbarium of the University of Michigan, (NY) Herbarium of the New York Botanical Garden, (NYS) Herbarium of the New York State Museum, (T) Herbarium of the University of Tennessee, (UC) Herbarium of the University of California.

This paper gives the results of the study of the brown-spored species of the Geoglossaceae which belong to the genera *Geoglossum* and *Tricho-*

[1] Paper from the Herbarium and the Department of Botany of the University of Michigan. Published as excess pagination from funds of the Herbarium.

glossum. They are very closely related. *Trichoglossum* is separated from *Geoglossum* on account of the occurrence of setae in the hymenium and on the stipe. The species of the genus *Glocoglossum*, which was proposed by Durand, are treated in *Geoglossum*.

In the classification of species of *Geoglossum* and *Trichoglossum* the septation of the ascospores has generally been given considerable emphasis. In some species the ascospores are regularly 7-septate, in others they are regularly 15-septate and in others there is considerable variability in septation. In the regularly-septate species, the first septum divides the immature spore into two equal cells. Both cells then divide equally giving 4 cells and 3 septa. Equal divisions of each cell then follow giving 8 cells and 7 septa. This terminates the septation in the 7-septate species such as *Geoglossum simile*. In the 15-septate species equal divisions of each of the 8 cells produce a 15-septate, 16-celled spore. This is here designated as a 1–3–7–15 sequence in septation. Deviations from the 7- or 15-septate condition may be caused by irregular failures of septa to form, often evident as longer cells, or there may be divisions of one or more cells beyond the 7- or 15-septate conditions. In some species, such as *Geoglossum simile*, there are few such deviations. In others there may be considerable variation within the species in the number and extent of such deviations, as shown for the various varieties of *T. hirsutum*.

In some species variability in septation occurs which apparently is not due to deviations from the 7- or 15-septate conditions. Sinden and Fitzpatrick (1930) have concluded that the method of septation in *Trichoglossum velutipes*, *T. wrightii* and *T. variabile* is fundamentally different from that in *T. hirsutum*. They state that following the first septum, which divides the spore into two equal cells, four septa are formed apparently simultaneously dividing each cell into three and giving 6 cells and 5 septa. Each cell may then divide equally giving 11 septa. Deviations from this are due to failures of septa to form or to a few cells dividing again. Nannfeldt (**1942**) agrees that these species have a different sequence of septation than *T. hirsutum* but disagrees concerning the method. He states that the first septum is median and then the division of the two daughter cells is unequal giving 3 septa with four cells, the two terminal being almost twice as long as the two central cells. Then the two long terminal cells divide, giving 6 cells and 5 septa. He concludes that in *T. farlowii* the spores are mature at this stage but in *T. variabile* the cells each divide once more resulting in 12 cells and 11 septa. This is here designated as a 1–3–5–11 sequence. Some data are presented under *T. farlowii* which support Nannfeldt's hypothesis.

However, septation in *T. farlowii* may stop at any stage and mature spores may be 0-, 1-, 3- or 5-septate and irregularities produce a few 2-, 4-, 6- and 7-septate spores. In *T. velutipes, T. variabile* and *T. rasum* (*T. wrightii*) the septation may have the sequence suggested by Nannfeldt but the variations are so great that the sequence is obscured. However, a comparison of TABLES VII and VIII with TABLE VI indicates that the method of septation is different than in *T. hirsutum*.

Not only the variability of septation but of the color of ascospores in some of the species is greater than indicated by Durand. He has attributed some of this to slowness in maturity. Thus in *G. alveolatum* he states that the ascospores are "at first continuous, then 7-, finally 15-septate" and are "for a long time hyaline but ultimately becoming pale brown or fuliginous." This statement implies that at maturity the ascospores are 15-septate and brown. However, discharged ascospores have been found to be 0–15-septate and hyaline. Brown spores have been seen in only a few asci. So far as possible in this investigation, ascospores have been studied from discharged spores obtained by dipping ascocarps in drops of water on the microscope slide. It is unlikely that further development in septation or color occurs in such spores. The variations which have been observed are considered those of mature spores. There is some possibility of error when mixed collections of species occur. However, such collections are usually easily detected in mounts of the hymenium. Studies of microscopic characters have mostly been made in mounts heated in lactophenol (Amman's medium). When the hymenium is agglutinated treatment with 5% potassium hydroxide was found necessary.

In this study 12 species of *Geoglossum* and 9 of *Trichoglossum* have been recognized from North America. The two genera are best represented in eastern North America, only six of the species having been seen from the west as compared with 20 from the east. One species is entirely tropical. Of the 833 North American specimens studied, 544 were species of *Geoglossum* and 339 were *Trichoglossum*. *Geoglossum simile* (293 specimens) was the most abundant species and *Trichoglossum hirsutum* (161 specimens) was next in prevalence.

Hymenium without acuminate brown setae.....*Geoglossum*
Hymenium with numerous, acuminate, projecting brown setae.....*Trichoglossum*

GEOGLOSSUM Pers. ex Fr. Syst. Myc. **1**: 487. 1821

Gloeoglossum Durand, Ann. Myc. **6**: 418. 1908.

Ascocarps usually clavate, compressed above, black or brown, fleshy, dry, viscid or gelatinous, especially in wet weather, with the hymenium

confined to the upper portion of the ascocarp; stipes slender, terete, smooth, pubescent, squamulose or tufted setose-hirsute; asci clavate, inoperculate, usually 8-spored; ascospores clavate, subcylindric or subfusoid, brown, both brown and hyaline or in *G. alveolatum* most or all hyaline, septate or both septate and non-septate, paraphyses intermixed with the asci, in some species occurring on the stipes in modified forms scattered or united as small scales or tufts or as a continuous gelatinous layer.

Lectotype: *Geoglossum glabrum* Pers. ex Fr.

For *Geoglossum glutinosum*, *G. affine* and *G. difforme*, which have paraphyses in a continuous gelatinous layer on the stipe, Durand established a genus *Gloeoglossum*. Imai (**1942**) has concurred in this separation but considers that the genus was previously named *Cibalocoryne* by Hazslinszky. The validity of *Cibalocoryne* as a generic name is questionable, since Hazslinszky (**1881**) appears to have proposed it as a subgenus and only suggested a future generic status. Nannfeldt has concluded that the species of *Gloeoglossum* are more closely related to other species of *Geoglossum* than to each other and has included them in *Geoglossum* and this treatment is followed here.

All of the species of this study have had 8-spored asci with the exception of one variety of *G. nigritum* in which the asci were found to be 2–8-spored, mostly 4- and 8-spored. A much greater variation occurs in the ascospores. In most species the ascospores are brown when discharged. In three species, *G. fallax*, *G. alveolatum* and *G. intermedium*, both hyaline and light brown spores are produced. *G. alveolatum* is anomalous in that most of the spores are hyaline. The sequence of septation in the species appears to be 0–1–3–7–15 and they can be divided into two groups according as to whether 7 or 15 is the maximum septation. The species differ considerably in the number of septa. In some the septation is remarkably constant while in others it is very variable. In the latter, the variations appear to be due partly to septation terminating in some of the spores at the 1, 3 or 7 stages and partly to irregular omissions of septa. This variability finds its greatest expression in *G. fallax*, *G. intermedium* and *G. alveolatum*. *G. fallax* not only produces both hyaline and brown spores but also has 0–13-septate spores. All of the hyaline spores are nonseptate. In a number of the collections most of the brown spores are also non-septate and no definite sequence of septation is apparent for the others. *G. intermedium* and *G. alveolatum* have variations in septation in both hyaline and brown spores. As discussed under the species there is also considerable variation in the paraphyses.

The species also show differences in prevalence and distribution in North America. Collections of *G. affine, G. cohaerens, G. simile, G. difforme, G. pumilum, G. pygmaeum, G. alveolatum* and *G. intermedium* have been seen only from eastern North America. *G. glutinosum, G. nigritum, G. glabrum* and *G. fallax* occur in both eastern and western North America. *G. simile* is by far the most prevalent species, more than half of the specimens of *Geoglossum* studied being that species. Following in order of prevalence are *G. nigritum, G. difforme, G. glutinosum, G. fallax, G. glabrum, G. alveolatum,* and *G. intermedium. G. affine, G. cohaerens* and *G. pumilum* are known only from one or several specimens.

KEY TO SPECIES OF GEOGLOSSUM

1. Ascospores brown.....2
1. Ascospores both hyaline and brown; sometimes most or all hyaline.....10
2. Ascospores 7-septate or, if variable in septation, very rarely more than 7-septate.....3
2. Ascospores 15-septate or, if variable in septation, many with more than 7 septa.....8
3. Paraphyses continuing down the stipe forming a gelatinous layer.....4
3. Paraphyses not forming a continuous gelatinous layer on the stipe.....5
4. Ascospores (55–)60–90(–100)μ long, subcylindric.....*G. glutinosum*
4. Ascospores mostly less than 60μ long, narrowly clavate.....*G. affine*
5. Paraphyses usually strongly curved above, sparsely or moderately septate, cylindric, not constricted at septa, the upper cell not or moderately enlarged...6
5. Paraphyses straight or somewhat curved above, closely septate in the upper portions, more or less constricted at septa, the upper cells variously enlarged.....7
6. Paraphyses strongly agglutinated by dark amorphous matter, ascospores subfusoid or subcylindric, 3–4-septate.....*G. cohaerens*
6. Paraphyses not agglutinated by dark amorphous matter, ascospores clavate, 7-septate or, if variable in septation, many 7-septate.....*G. nigritum*
7. Paraphyses having upper cells variously enlarged, globoid, ellipsoid or obovoid, ascospores (45–)55–78(–90)μ long; stipes smooth.....*G. glabrum*
7. Paraphyses having many two-celled, oblong or barrel-shaped segments, ascospores (60–)75–90(–105)μ long; stipes usually squamulose.....*G. simile*
8. Paraphyses continuing down the stipe forming a gelatinous layer...*G. difforme*
8. Paraphyses not forming a continuous layer down the stipe.....9
9. Ascospores (85–)90–130(–145)μ long.....*G. pumilum*
9. Ascospores (120–)125–160(–180)μ long.....*G. pygmaeum*
10. Hyaline spores non-septate, paraphyses not agglutinated by amorphous matter, stipes squamulose.....*G. fallax*
10. Hyaline spores frequently septate, paraphyses agglutinated by amorphous matter, stipes tufted setose-hirsute.....11
11. Ascospores (50–)67–82(–95)μ long, hyaline spores 0–15-septate..*G. alveolatum*
11. Ascospores (40–)50–65(–75)μ long, hyaline spores 0–7-septate..*G. intermedium*

GEOGLOSSUM GLUTINOSUM Pers. ex Fr. Syst. Myc. **1**: 489. 1821. (FIGS. 1, 2)

Geoglossum glutinosum Pers. Obs. Myc. **1**: 11. 1796.

Geoglossum viscosum Pers. ex Fr. Syst. Myc. **1**: 489. 1821.

Cibalocoryne viscosulum Hazsl. M. T. Akad. Ert. Term. Kör. **11**: 8. 1881.

Gloeoglossum glutinosum (Pers. ex Fr.) Durand, Ann. Myc. **6**: 419. 1908.

Cibalocoryne [as *Cibarocoryne*] *gelatinosa* (Pers. ex Fr.) Imai, Bot. Mag. **56**: 525. 1942.

Type: In the Persoon herbarium at Leiden, according to Durand.

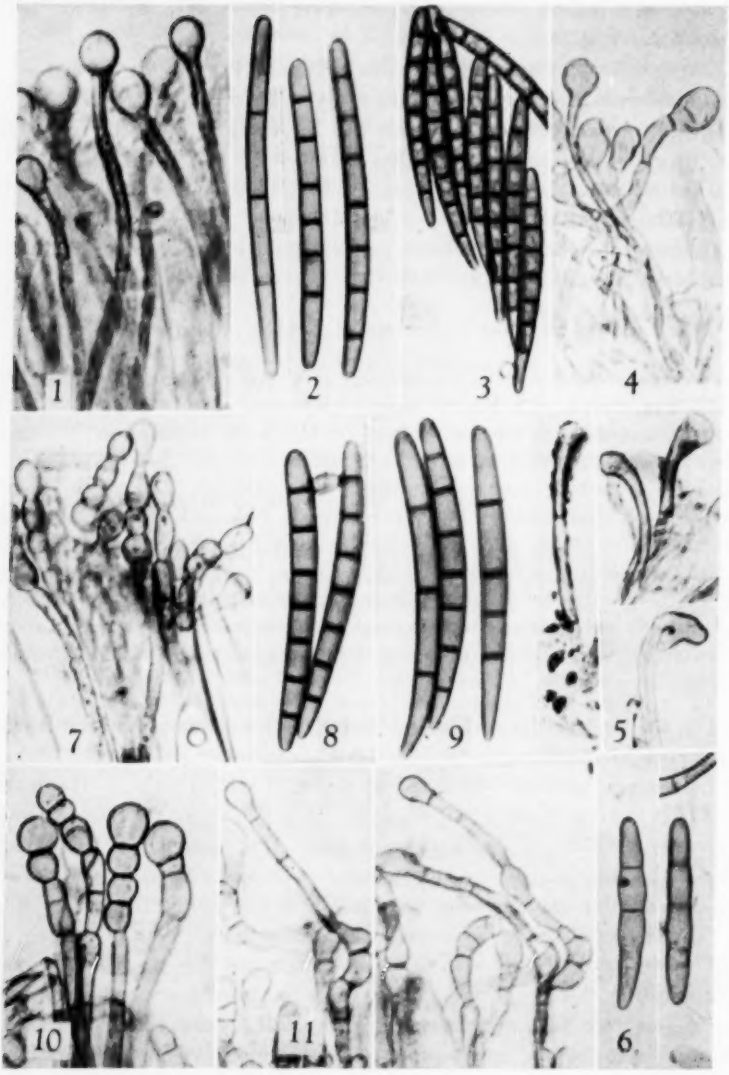
Ascocarps scattered to caespitose, very viscid, becoming gelatinous when wet, clavate, 1.5–5.5 cm long; ascogenous portion black, $\frac{1}{4}$ to $\frac{1}{3}$ the length, compressed, clavate, oblong or ellipsoid, 3–6 mm wide; stipes brown to brownish black, terete, 2–3 mm thick; asci clavate, 175–250 × 12–14 μ, 8-spored; ascospores subcylindric, slightly narrowing toward one or both ends, straight to slightly curved, (55–)60–90(–100) × 4–5 μ, brown, 0–7-septate, many collections mostly 3-septate, other collections mostly 7-septate; paraphyses hyaline below, light to dark brown above or entirely dark brown, straight to somewhat curved above, narrowly cylindric below, sparsely septate, with the terminal cells pyriform, broadly obovoid or subgloboid, continuing down the stipe in a thick gelatinous layer.

On soil on hillsides and in wet places, under cedar and in hardwoods. Collected in Michigan from July 13 to September 23.

Specimens studied: 40 from Michigan (MICH), New Hampshire (MICH), New York (MICH, NY, CUP), Oregon (MICH), Pennsylvania (MICH, NY), Tennessee (MICH), Virginia (NY), Puerto Rico (NY).

This species has also been reported from Europe and eastern Asia.

The ascospores are very variable in septation. Durand states that the spores of the type are mostly 1–3-septate but that in most collections the majority are 7-septate. The species has been described as 3-septate or 3–7-septate (see summary by Imai, **1941**). As given in TABLE I, nine collections were studied for the septation of ascospores. Six collections had 3-septate spores predominating. One had 3-septate and 7-septate approximately equal in number. In two collections almost all the spores were 7-septate. The septating therefore apparently follows a 1–3–7 sequence. The 2-, 4-, 5- and 6-septations (FIG. 2) are due to irregular omissions of septa as indicated by cells which are longer than



FIGS. 1, 2. *Geoglossum glutinosum;* 1, paraphyses; 2, three ascospores 3-, 6- and 7-septate. 3, 4. *Geoglossum affine,* type; 3, seven 7-septate ascospores; 4, paraphyses. 5, 6. *Geoglossum cohaerens,* type; 5, paraphyses; 6, two 3-septate ascospores. 7, 8. *Geoglossum glabrum* var. *glabrum;* 7, paraphyses; 8, two 7-septate ascospores. 9. *Geoglossum glabrum* var. *heterosporum,* type, 3-, 7- and 4-septate ascospores. 10. *Geoglossum glabrum* var. *americanum,* type, paraphyses. 11. *Geoglossum glabrum* var. *elongatum,* type, paraphyses. All × 600.

TABLE I

VARIATION IN THE SEPTATION OF DISCHARGED ASCOSPORES OF GEOGLOSSUM GLUTINOSUM EXPRESSED IN PERCENTAGES[2]

Collection[3]	Number of septa							
	0	1	2	3	4	5	6	7
Smith, *28189*	0	2	4	87	1	2	1	3
Mains, *4207*	8	3	2	83	0	0	1	3
Smith, *33579*	1	3	1	89	+	0	+	6
Imshaug, *3469*	3	14	4	64	1	0	0	14
Smith, *33042*	2	0	0	64	5	5	9	15
Smith, *33046*	0	1	0	47	8	19	16	8
Mains, *33–483*	0	1	0	42	2	5	10	40
Mains, *33–526*	0	0	0	1	1	1	2	95
Smith, Aug. 1929	0	0	0	0	0	1	3	96

the others. The species apparently is potentially 7-septate. Whether the predominantly 3- and 7-septate conditions should be recognized as forms is doubtful.

GEOGLOSSUM AFFINE (Durand) Lloyd, Myc. Writings **5**, Geoglossaceae, p. 9. 1916. (FIGS. 3, 4)

Glocoglossum affine Durand, Ann. Myc. **6**: 420. 1908.

Cibalocoryne [as *Cibarocoryne*] *affine* (Durand) Imai, Bot. Mag. **56**: 526. 1942.

Type: Knoxboro, Oneida Co., N. Y., H. S. Jackson, in the Herbarium of the Department of Plant Pathology, Cornell University (CUP *338*).

Ascocarp black, clavate, 1.5–2.5 cm long, viscid to very gelatinous when wet; ascogenous portion ½ the length; stipes terete, slender, smooth; asci clavate, 130–160 × 15–18 μ, 8-spored; ascospores clavate (40–)45–62 × 5–6 μ, brown, mostly 7-septate, rarely fewer; paraphyses longer than the asci, hyaline to light brown, remotely septate below, closely septate above, with the lower cells cylindric, with one to several upper cells clavate, obovoid, ellipsoid or globoid, continuing down the stipe in a thick gelatinous layer.

Specimen studied: The type, on soil in swamp, Knoxville, New York, H. S. Jackson, Aug. 20, 1904.

The status of this species is very uncertain. The ascospores are shorter and more clavate than those of *G. glutinosum* and the paraphyses are more septate and irregular. In these characters it resembles *G.*

[2] + indicates that the number of spores was less than 0.5%.

[3] All collections in the Herbarium of the University of Michigan unless otherwise indicated.

glabrum. However, unlike that species, the paraphyses continue down the stipe in a gelatinous layer.

Durand cited another specimen in addition to the type. This (CUP *7980*) is a single broken ascocarp and differs in having longer ascospores (55–)60–80(–85)μ and paraphyses with only the terminal cells inflated (consult Durand fig. 74). The specimen is more in agreement with *G. glutinosum* except the paraphyses are more closely septate. There is another specimen (CUP *30739*) in the Herbarium at Cornell University under *Gloeoglossum affine*. It also is a single broken ascocarp. The ascospores agree in size with *G. affine* being (45–)53–62(–65)μ long and 7-septate but the paraphyses are curved at the apices resembling those of *G. nigritum*.

GEOGLOSSUM COHAERENS Durand, Ann. Myc. **6**: 430. 1908. (FIGS. 5, 6)

Lectotype: Newfield, New Jersey, Dec. 1, 1889,[4] in the Herbarium of the New York Botanical Garden.

Ascocarps black, clavate, 2–4.5 cm long; ascogenous portion about $\frac{1}{2}$ of the length, clavate to narrowly ellipsoid; stipes terete, squamulose; asci clavate, 125–150 × 12–15 μ; 8-spored; ascospores subfusoid to subcylindric with rounded ends, (30–)38–49(–55) × 5–6 μ, light brown, 0–7-septate, mostly 3- or 4-septate, thin-walled; paraphyses slightly exceeding the asci, dark brown, moderately septate, straight to strongly curved, clavate, pyriform, irregular or contorted at the apices, agglutinated with dark amorphous matter to form an epithecium.

Specimens studied: Newfield, New Jersey, J. B. Ellis, Nov. 6, 1889[4] (NY and CUP, CI-*159*), Nov. 1, 1889[4] (NY and CUP, CI-*160*).

This species is apparently known only from the two specimens cited by Durand who states that they were in the Ellis collection of the New York Botanical Garden. Durand did not designate a type. The collection of December 1 is selected as the lectotype since it has more abundant mature asci. Portions of each collection are in the Herbarium of the Department of Plant Pathology of Cornell University and all have been available for this study.

Durand states that the ascospores are "for a long time hyaline, later becoming pale brown, continuous to 7-septate." He groups *G. cohaerens* with *G. intermedium* and *G. alveolatum* in which the discharged spores are both hyaline and brown. In a study of the specimens very few free spores were found and they were light brown. All the well-differentiated

[4] Published as 1899 by Durand.

spores in asci were light brown. This would indicate that the ascospores are probably brown at maturity but does not exclude the possibility that both hyaline and brown spores are discharged as in *G. intermedium* and *G. alveolatum*. On account of the absence of discharged spores it was not possible to determine with certainty the variation in septation of mature spores. Of 150 spores in asci and free in crushed mounts, 12 were without septa; 13, 1-septate; 6, 2-septate; 67, 3-septate; 35, 4-septate; 12, 5-septate; 4, 6-septate; 1, 7-septate.

Nannfeldt (**1942**) has suggested that *G. cohaerens* is closely related to or perhaps not distinct from *G. littorale* (Rostr.) Nannf. The latter is apparently known only from the type from Jutland, Denmark and was described as a *Leptoglossum* having 0–4-septate, hyaline ascospores 50–60 × 5 μ. Nannfeldt studied the type and found that the spores "take finally a faint grayish-brown colour." He therefore transferred the species to *Geoglossum*. Lind (**1913**) illustrates the paraphyses as filiform without enlarged apices. It is doubtful that it is the same as *G. cohaerens*. Nannfeldt has also noted that *G. elongatum* Starb. ex Nannf. from Sweden resembles *G. cohaerens* but differs in shape and color of the paraphyses and in the acute tufts of hairs on the stipes. It should be noted that *G. cohaerens* does have a few paraphyses of the type illustrated by Nannfeldt for *G. elongatum*.

G. vleugelianum Nannf. appears to be very closely related to *G. cohaerens*. A specimen in the Herbarium of the Department of Plant Pathology, Cornell University (CU *6441*) is apparently that sent to Durand by Vleugel and appears to be a part of the type collection as given by Nannfeldt. It is labeled *Geoglossum difforme* with *difforme* crossed out and *nigritum* written above. The data on the packet read Suecia Umeå 64° nord. lat. Sept., 1902, J. Vleugel. As stated for the type by Nannfeldt the packet contains a mixture of species. It is difficult therefore to be certain concerning the material. A broken stipe was found to bear scales similar to those for *G. cohaerens*. The principal difference from the latter species is longer ascospores (50–65 μ).

GEOGLOSSUM NIGRITUM (Fr.) Cooke, Mycographia **1**: 205. 1879.
Clavaria nigrita Fr. Syst. Myc. **1**: 483. 1821.

Type: Uppland, Halmbyboda, Sweden, 1853, E. P. Fries, in Fries' Herbarium, Uppsala, cotype in Kew Herbarium according to Nannfeldt.

Ascocarps scattered or in small groups, clavate, very variable in size, 5–70 mm long; ascogenous portion ¼ or ½ the length, somewhat compressed, 1–5 mm wide, dark brown to black; stipes slender, terete, 0.5–

2.0 mm thick, sometimes somewhat viscid, glabrous, minutely pubescent, furfuraceous or squamulose, wood brown to black; asci clavate, 125–180 × 16–20 μ, mostly 8-spored, sometimes less; ascospores clavate, 30–90 × 4.5–6.5 μ, straight or somewhat curved, dark brown, paraphyses hyaline to brown, longer than the asci, straight or strongly curved above, cylindric, not or slightly enlarged at apex, the terminal cell cylindric, narrowly obovoid or clavate.

A. G. NIGRITUM var. **nigritum.** (FIGS. 14, 15)

Asci 8-spored; ascospores (40–)60–78(–90) × 4.5–6.5 μ, mostly 7-septate.

Very variable in habitats, occurring among sphagnum and other mosses, on soil in well drained areas and on wet soil by streams and in bogs. Collected in Michigan from July 30 to September 24.

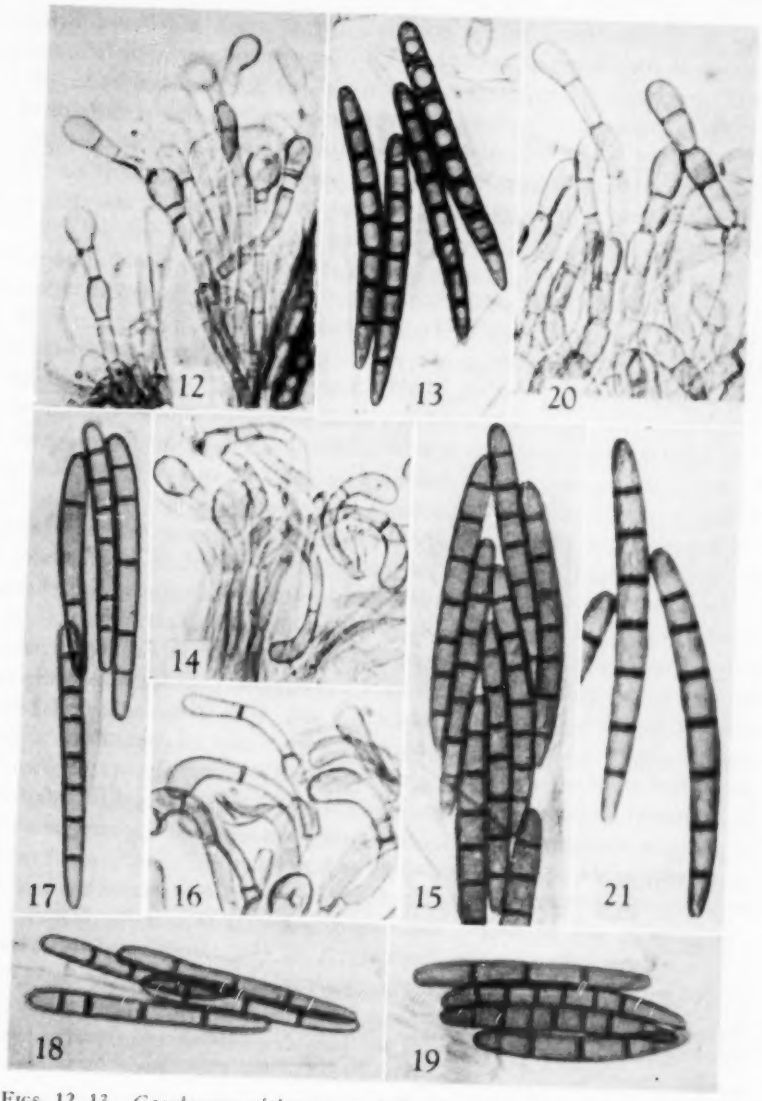
Specimens studied: 71 from California (UC), Connecticut (UC), Idaho (MICH), Maine (NY), Massachusetts (NYS), Michigan (MICH), New York (CUP, NYS, NY, MICH), Oregon (MICH), Pennsylvania (MICH, NY), Tennessee (MICH), Virginia (NY), Washington (UC, MICH), Bermuda (NY, UC).

B. G. NIGRITUM var. **heterosporum** var. nov. (FIGS. 16–19)

Var. *nigrito* simile sed ascis 2-8-sporis; ascosporis (30–)42–63(–80) × 5–6 μ, 1–7-septatis.

On conifer log, Mackinaw City, Michigan, July 31, 1949, A. H. Smith, *32820,* type.

There is considerable confusion concerning the application of the name *Clavaria nigrita.* Fries in his treatment in the Systema refers to *C. nigrita* of Persoon. Bourdot and Galzin (**1927**) and Coker (**1923**) include *Clavaria nigrita* Pers. in their treatments of the genus *Clavaria.* Corner (**1950**), on the other hand, in his recent monograph considers that *Clavaria nigrita* as treated by Fries is a *Geoglossum.* Nannfeldt (**1942**) under *Geoglossum nigritum* Cooke gives as a synonym "*Clavaria nigrita* Fr. Hym. Eur. p. 676 (1874) (non Pers. vix, Fr. Sys. Myc. et Epicr.)." Cooke, when he described and illustrated "*Geoglossum nigritum* Pers.," cited *Clavaria nigrita* Pers. Syn. p. 604 and Fries Epicr. 1, p. 578. He states that the species was figured from specimens in the herbarium of Berkeley communicated by Professor Fries. Apparently he accepted this collection as authentic for *Clavaria nigrita.* It appears to be the collection to which Fries refers in his treatment of *Clavaria nigrita* in the Hymenomycetes Europaei. He may have included two



FIGS. 12, 13. *Geoglossum glabrum* var. *inflatum*, type; 12, paraphyses; 13, four 7-septate ascospores. 14, 15. *Geoglossum nigritum* var. *nigritum;* 14, paraphyses; 15, 7-septate ascospores from an 8-spored ascus. 16–19. *Geoglossum nigritum* var. *heterosporum*, type; 16, paraphyses; 17–19, ascospores with 2- to 7-septations from 4-spored asci. 20, 21. *Geoglossum simile*, type; 20, paraphyses; 21, two 7-septate ascospores. All × 600.

fungi in his treatment in the Systema. If *Clavaria nigrita* of Fries' Systema is considered as applying to a *Clavaria* and not a *Geoglossum* it will be necessary to give a new name to the species described by Cooke. However, since the collection of *"Clavaria nigrita"* in the herbarium of Fries appears to be the same as that from which Cooke derived his description of *Geoglossum nigritum,* it seems best to accept it as the type of *Clavaria nigrita* unless further information can be presented to the contrary.

Geoglossum nigritum is very closely related to *G. simile* and *G. glabrum,* which differ in having paraphyses that are closely septate above with the cells variously enlarged and more or less constricted at the septa. *Geoglossum barlae* Boud. appears also to differ mostly in the type of paraphyses. Boudier (**1888**) described and illustrated them as having the upper portion twisted in a loose irregular spiral or corkscrew. Nannfeldt reports a specimen from the Bresadola herbarium as *G. barlae,* for which the paraphyses are described as crozier-like and illustrated as ending in a tight coil forming a head.

Nannfeldt has emphasized the variability of *G. nigritum* in spore length. He reports extremes having mean lengths of 58.3 and 86.9 μ. In the collections reported here somewhat similar differences have been noted (M 60.4–M 78.3 μ) for the variety *nigritum.* Nannfeldt has also stated that collections made from the same locality in different years showed considerable differences in spore length (M 68.5–M 86.8 μ), possibly indicating environmental effect on spore size. As noted by Durand there is also considerable variation in size of ascocarps. The stipes also may be minutely pubescent due to scattered paraphyses or furfuraceous or minutely squamulose due to the adherence of paraphyses in plates to form small scales. Some collections which appear glabrous have paraphyses on the stipe which are matted and closely appressed, probably due to weathering. The stipes are sometimes somewhat viscid, especially in wet weather. There is a specimen (CUP *30739*) under *Gloeoglossum affine* in the Herbarium of the Department of Plant Pathology of Cornell University having paraphyses similar to *G. nigritum,* which form a thin gelatinous layer on the stipe. Unfortunately there is only one broken ascocarp. This suggests that there may be a species closely related to *G. nigritum* having the glutinous *Gloeoglossum* condition.

Cooke illustrated the ascocarps of *G. nigritum* as black, as is indicated by the specific name. Durand describes them as black or brownish black. Some of the Michigan collections are decidedly brown in the fresh condition. Notes for fresh ascocarps by A. H. Smith give the ascogenous portion as dark brown or mummy brown and the stipe as

wood brown. The dried specimens are darker in color. They do not differ otherwise from the typical condition.

The paraphyses furnish the most important diagnostic characters for the separation of this species from others in the *G. glabrum* group. Cooke illustrates the paraphyses as erect and cylindric with the terminal cell somewhat enlarged. Nannfeldt, who has studied the type, includes the species in his key with those which have paraphyses strongly curved. Usually the paraphyses are mostly strongly curved above the asci. They are cylindric without terminal enlargement or more frequently with the terminal cell enlarged. There is little or no constriction at the septa.

Most collections of *G. nigritum* have asci which are 8-spored with ascospores 7-septate, rarely less. A rather marked deviation from this condition occurs in the variety *heterosporum* in which both 4- and 8-spored asci occur. Eight-spored asci are the most abundant. Asci with four spores are common and occur in the same hymenium with the 8-spored. Other numbers of spores have been observed occasionally. Of 395 discharged ascospores 1% was 1-septate; 1%, 2-septate; 42%, 3-septate; 10%, 4-septate; 6%, 5-septate; 9%, 6-septate; 31%, 7-septate. There appears to be a tendency for the 8-spored asci to have more 7-septate and longer spores than the 4-spored asci.

Nannfeldt has described a species *Geoglossum montanum* which he states approaches *G. nigritum*, but the asci are 3–6-spored and hardly ever produce 8 spores. Apparently there is little or no variation in the septation of the ascospores since he states that they are commonly 7-septate. *G. nigritum* var. *heterosporum* appears to be intermediate between typical *G. nigritum* and *G. montanum*.

Another variant has been found in a collection (UC *532331*) under *G. nigritum* in the Herbarium of the University of California. The collection is a mixture of *G. nigritum* and *G. glabrum*. One ascocarp differs from others of *G. nigritum* in having 1–7-spored asci with 4-spored asci common. The ascospores are very variable in length, 35–120 μ. Most of the spores are clavate but a few occur which are very abnormal in shape. While 7-septate spores are common, the septation varies from 0–10. The paraphyses are similar to those of *G. nigritum*.

GEOGLOSSUM GLABRUM Pers. ex Fr. Syst. Myc. **1**: 488. 1821.

Lectotype: In Persoon Herbarium, Rijks Herbarium, Leiden, according to Durand.

Ascocarps scattered to crowded, black, clavate, 1.5–10.0 cm long; ascogenous portion $\frac{1}{3}$ to $\frac{1}{2}$ of the length, compressed, 3–8 mm wide;

stipes terete, 1–4 mm thick, smooth; asci clavate, 150–190 × 20 μ, 8-spored; ascospores clavate, (45–)55–78(–90) × 6–8 μ, straight or somewhat curved, dark brown; paraphyses exceeding the asci, straight or somewhat curved, above, hyaline or light brown below, light to dark brown and closely septate above, the upper cells variously enlarged (see the following varieties).

A. G. GLABRUM var. **glabrum.** (FIGS. 7, 8)
Geoglossum cookeianum Nannf. Ark. f. Bot. **30A**: 22. 1942.

Ascospores mostly 7-septate, rarely less; paraphyses having upper cells variously enlarged, globoid, ellipsoid and obovoid, moderately constricted at the septa (FIG. 7).

On soil. Collected in Michigan in August and September.

Specimens studied: Kentucky, Lois L. Eubank (UC *706928*). Michigan, H. A. Imshaug *4903, 5069* in part (MICH); A. H. Smith *15318, 37337* in part, *37542, 37548, 39758, 39770* (MICH). New Hampshire, W. C. Sturgis (NY). Tennessee, A. J. Sharp (T *4276*).

B. G. GLABRUM var. **heterosporum** var. nov. (FIG. 9)

Var. *glabro* simile sed ascosporis 1–7-septatis.

On soil in low moist places, Cabin John, Maryland, C. H. Kauffman, Aug. 19, 1919, type (MICH).

This variety differs from others of *G. glabrum* in having ascospores with variable septation. The discharged ascospores were found to be 4%, 1-septate; 2%, 2-septate; 13%, 3-septate; 5%, 4-septate; 5%, 5-septate; 3%, 6-septate; 68%, 7-septate.

C. G. GLABRUM var. **americanum** var. nov. (FIG. 10)

Var. *glabro* simile sed paraphysibus sursum pauci- magnis globosis cellis.

On soil. Collected in Michigan July 29 and Aug. 6.

Specimens studied: Michigan, A. H. Smith *6683*, type (MICH); A. H. Povah Fp *417* (MICH). Washington, A. H. Smith *17939* (MICH).

Variety *americanum* differs from var. *glabrum* in having a few of the upper cells of the paraphyses decidedly globose and strongly constricted at the septa (FIG. 10). It is very similar to *G. glabrum* sensu Nannf.

D. G. GLABRUM var. **inflatum** var. nov. (FIGS. 12, 13)

Var. *glabro* simile sed paraphysibus sursum dense septatis non constrictis ad septas et inflatis summis cellis (FIG. 12).

In sphagnum and other mosses and on soil. Collected in Michigan from July 20 to September 15.

Specimens studied: Michigan, C. H. Kauffman and L. E. Wehmeyer (MICH); A. H. Smith *10951*, type (MICH). New York, S. H. Burnham (NYS). Virginia, W. H. Long *2257* (NY).

Usually only the terminal cells of the paraphyses of this variety are enlarged. They are obovoid or pyriform, rarely ellipsoid or globoid. Below the terminal cells the upper cells of the paraphyses are mostly short cylindric. There is little or no constriction at the septa (FIG. 12). Whether this variety belongs here or in *G. nigritum* is questionable but is included in *G. glabrum* on account of the straight, closely septate paraphyses.

E. G. GLABRUM var. **elongatum** var. nov. (FIG. 11)

Var. *glabro* simile sed paraphysibus longis, cellis cylindricis et inflatis immixtis.

On soil.

Specimens studied: California, A. H. Smith *3797*, *9378* type, *9387* (MICH). Washington, A. H. Smith *18016* (MICH).

In this variety the paraphyses are considerably elongated and successions of cylindric cells are interspersed with inflated cells giving a proliferated appearance (FIG. 11).

It is not possible to determine with certainty what Persoon and Fries included in *Geoglossum glabrum* from their descriptions. Durand, who studied specimens from the Persoon herbarium, has illustrated asci, ascospores and paraphyses of a specimen which he selected as the type (**1908**, fig. 50). The ascospores are figured as 7-septate and the paraphyses as terminating with chains of a few somewhat enlarged cells. Van Luyk (**1919**) reports that two other species, *Geoglossum fallax* and *Trichoglossum hirsutum*, are also included under *G. glabrum* in the Persoon herbarium.

There is considerable disagreement concerning the specific limitations of *Geoglossum glabrum*. In the widest concept, it is a species having clavate, 7-septate ascospores and considerable variation in the paraphyses. Durand includes *G. simile* Peck and *G. difforme* sensu Cooke. He recognizes *G. nigritum* as a closely related species and states that it has "remotely septate paraphyses which are slender and thickened only in the terminal cell" and that *G. glabrum* has "closely septate commonly

more or less moniliform paraphyses" which show considerable variability. Nannfeldt, in addition to *G. nigritum*, recognizes *G. simile* and *G. cookeianum* (*G. difforme* sensu Cooke) as species distinguished from *G. glabrum* by their paraphyses. Imai has also described a species, *G. japonicum*, which is separated mostly by type of paraphyses.

Through the kindness of Dr. J. A. Nannfeldt specimens determined as *G. glabrum* and *G. cookeianum* by him have been available for study and various exsiccati cited by him have been examined. The type of *G. simile* in the herbarium of the New York State Museum has been studied. In agreement with Nannfeldt it has been concluded that *G. simile* is a separate, closely related species which can be distinguished with considerable accuracy. The paraphyses separate easily. The moniliform upper portions of the paraphyses contain many two-celled segments (FIG. 20). The ascospores are (60–)75–90(–105)μ long. The stipes are usually squamulose. Most of the collections from North America which have been determined as *G. glabrum* are *G. simile*.

As treated by Nannfeldt, *G. glabrum* has paraphyses terminated by one to a few, dark brown, ellipsoid or globoid cells which sometimes reach a diameter of 15μ. They adhere very strongly. He gives the length of the ascospores as (55–)65–80(–90)μ and the stipes as smooth. He states that this species grows exclusively in Sphagna in Europe and gives *G. sphagnophilum* Ehrenb., *G. sphagnorum* Pers. ex Maug. & Nestl. and *G. ophioglossoides* var. *sphagnophilum* Rehm as synonyms. *Geoglossum cookeianum* as distinguished by Nannfeldt has paraphyses which adhere less strongly and have longer chains of enlarged cells which vary considerably in size and shape. Other variants have been found in the North American collections. Usually there is considerable variation in the types of paraphyses within collections and distinctions are based on the predominance of types. It is therefore not surprising that there is disagreement in treatments. It seems best to recognize these variants as varieties of *G. glabrum*.

Geoglossum cookeianum Nann. has paraphyses similar to those illustrated by Durand for the type of *G. glabrum*. The substratum for the type is not known. Fries recognized a variety *sphagnophilum* (l.c.) for collections in sphagnum and otherwise apparently considered that the species grew on soil. *G. cookeianum* is considered a synonym of *G. glabrum* var. *glabrum* as treated here. The European collections on sphagnum accordingly are considered a variety, *G. glabrum* var. *sphagnophilum* (Ehrenb.) Fr. It differs from variety *americanum* in having paraphyses more agglutinated and in being limited to sphagnum. *G. japonicum* should also probably be considered a variety of *G. glabrum*.

GEOGLOSSUM SIMILE Peck, Buff. Soc. Nat. Sci. **1**: 70. 1873. (FIGS. 20, 21)

Type: Sandlake, N. Y., Chas. H. Peck, Herbarium New York State Museum.

Ascocarps scattered to caespitose, brownish black or black, clavate, very variable, 2–7 cm long; ascogenous portion $\frac{1}{8}$ to $\frac{1}{2}$ of the length, compressed, 2–12 mm wide; stipes terete, 1–5 mm thick, usually squamulose, especially above, sometimes minutely pubescent or smooth; asci clavate, 170–200 × 20–22 μ, 8-spored; ascospores clavate, straight or frequently somewhat curved, (60–)75–90(–105) × 6–9 μ; dark brown, 7-septate, rarely with fewer septa; paraphyses exceeding the asci, straight or somewhat curved above, closely and abundantly septate above, with the upper cells not or slightly enlarged, constricted at septa to form many oblong, ellipsoid or obovoid 2-celled segments.

In swamps, bogs and well drained soils. On humus, rotten wood and in sphagnum and other mosses. In Michigan this species has been collected from June 30 to Sept. 24.

Specimens studied: 293 from Connecticut (UC, NY), Delaware (NY), Maine (MICH, NY), Massachusetts (MICH, NY), Michigan (MICH), New Hampshire (MICH, NY), New Jersey (MICH, NY), New York (MICH, NYS, NY, UC), North Carolina (T), Rhode Island (NY), Pennsylvania (NY), Tennessee (T), Virginia (T), West Virginia (T), Wisconsin (NY), New Brunswick (MICH), Nova Scotia (MICH), Ontario (MICH, UC, NY), Quebec (MICH, NY).

The separation of *G. simile* from *G. glabrum* has been discussed in connection with the latter species. The squamulose stipes of *G. simile* are due to the occurrence of paraphyses on the stipes which adhere together and form plates. In a few collections the paraphyses are scattered and the stipes are somewhat pubescent. Also occasionally the paraphyses are appressed or absent, probably due to weather conditions, and the stipes appear smooth.

GEOGLOSSUM DIFFORME Fr. Syst. Myc. **1**: 489. 1821.
Geoglossum peckianum Cooke, Hedwigia **14**: 10. 1875.
Gloeoglossum difforme (Fr.) Durand, Ann. Myc. **6**: 421. 1908.
Cibalcoryne [*Cibarocoryne*] *peckianum* (Cooke) Imai, Bot. Mag. **56**: 565. 1942.

Type: Småland, Sweden, E. P. Fries, in the herbarium of Fries at Uppsala according to Durand.

Ascocarps scattered to crowded, black, clavate, 3–12 cm long, viscid to gelatinous, especially in wet weather; ascogenous portion compressed,

clavate to oblong, about $\frac{1}{4}$ to $\frac{1}{2}$ the length, 3–14 mm wide; stipes terete, up to 6 mm thick; asci clavate, 240–275 × 18–25 μ, 8-spored; ascospores fusoid-clavate, (70–)92–115(–135) × 6–7 μ, brown, 15-septate or 5–15(–17)-septate, paraphyses brownish, longer than the asci, slender, cylindric, septate, much twisted and coiled above forming a dense epithecium, continuing down the stipe forming a thick gelatinous layer.

A. G. DIFFORME var. **difforme.** (FIGS. 22, 23)

Ascospores mostly 15-septate.

On wet and dry soils and rotting logs. In Michigan collected from August 1 to September 19.

Specimens studied: 46 from Alabama (NY), Indiana (NY), Maine (MICH, NY), Massachusetts (MICH, NY), Michigan (MICH), Mississippi (NY), New Hampshire (MICH, NY), New York (MICH, NY, UC), North Carolina (MICH, T), Pennsylvania (MICH, NY), South Carolina (NY), Tennessee (MICH, T, UC), Vermont (NY), Virginia (T, UC, NY), West Virginia (T), Nova Scotia (MICH), Ontario (NY), Quebec (MICH, NY).

B. G. DIFFORME var. **variabile** var. nov. (FIG. 24)

Var. *difforme* simile sed ascosporis 5–15(–17)-septatis.

On soil in hardwoods.

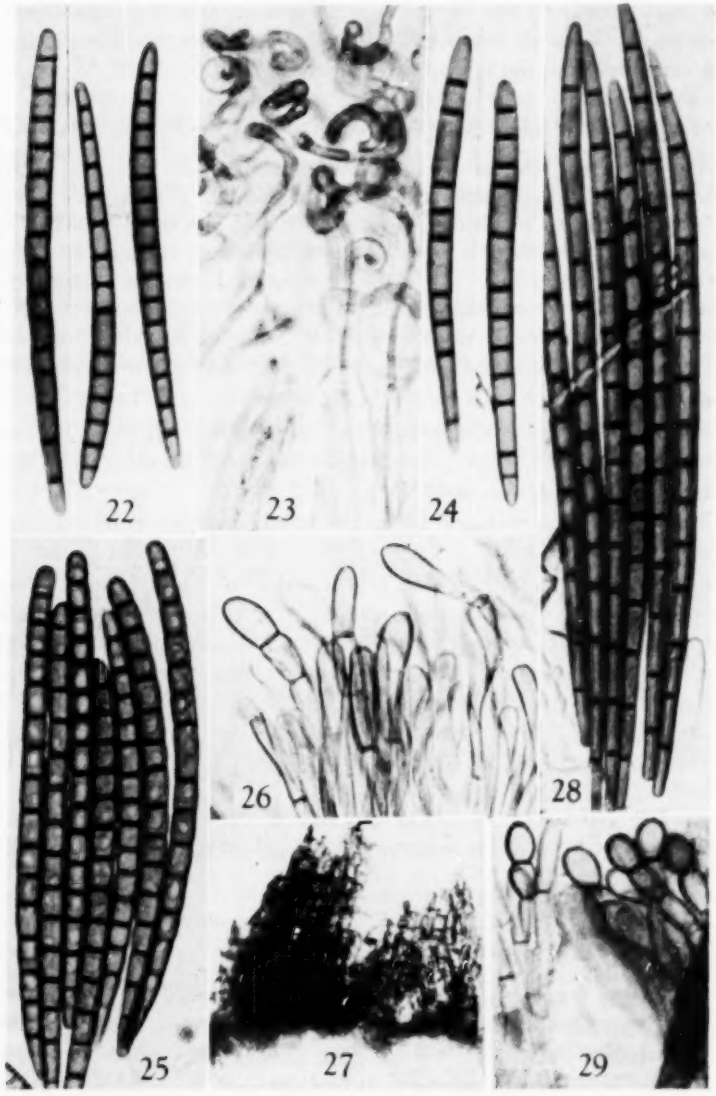
Specimens studied: Michigan, H. A. Imshaug *4907*; A. H. Smith *33214* type, *35874*, *37664* (all MICH); Tennessee, L. R. Hesler (T *8133*); A. H. Smith *9671*, *10190* (MICH).

In most collections of *G. difforme* the discharged ascospores are all 15-septate or with a few spores with less septa. In some collections a considerable variation in septation was found, as shown in TABLE II.

TABLE II

VARIATION IN THE SEPTATION OF DISCHARGED ASCOSPORES OF GEOGLOSSUM DIFFORME VAR. VARIABLE EXPRESSED IN PERCENTAGES

Collection[a]	Number of septa												
	5	6	7	8	9	10	11	12	13	14	15	16	17
Smith *10190*	0	0	0	1	1	3	3	4	3	4	81	0	0
Smith *9671*	0	0	0	1	2	2	4	2	3	6	80	0	0
Smith *35874*	1	0	9	1	1	1	5	5	1	5	71	0	0
Hesler T *8133*	2	2	5	0	2	6	6	5	14	8	50	0	0
Smith *37664*	1	1	19	4	5	3	3	2	6	8	48	0	0
Imshaug *4907*	0	1	1	3	0	2	7	10	14	21	37	3	1
Smith *33214*	0	0	1	6	5	6	16	18	14	21	13	0	0



FIGS. 22, 23. *Geoglossum difforme* var. *difforme*; 22, two 15-septate and one 14-septate ascospores; 23, paraphyses. 24. *Geoglossum difforme* var. *variable*, type, 9- and 12-septate ascospores. 25–27. *Geoglossum pumilum*; 25, six 15-septate and one 14-septate ascospores; 26, paraphyses; 27, scales of the stipe × 300. 28, 29. *Geoglossum pygmaeum*; 28, 15-septate ascospores; 29, paraphyses. × 600 unless otherwise indicated.

The deviations from the 15-septate condition are due to irregular omissions of septa, as shown in FIG. 24. It seems desirable to recognize this variation from the typical as a variety.

GEOGLOSSUM PUMILUM Winter, Grevillea **15**: 91. 1887. (FIGS. 25–27)

Ascocarps few, clavate to subcapitate, small, 5–20 mm long, black; ascogenous portion oblong, ovoid or subgloboid, 1 mm wide; stipes slender, terete, 0.5 mm thick, squamulose; asci clavate, 175–200 × 20–22 μ, 8-spored; ascospores brown, cylindric-clavate, straight or slightly curved, (85–)90–130(–145) × 5–6 μ, mostly 15-septate; paraphyses longer than the asci, straight or slightly curved above, moderately brown above, lighter brown to hyaline below, the lower cells cylindric and the upper clavate, obovoid or ellipsoid, not or slightly constricted at the septa.

On soil.

Specimens studied: Virginia, W. H. Long *2246* (CUP). Bermuda, L. Ogilvie (CUP *33218*); Britton, Brown and Seaver *1364* (NY); Whetzel, Olive and Seaver (NY).

This species was collected by E. Ule no. *338* at São-Francisco, Brazil and was described by G. Winter (**1887**). Durand did not include it in his monograph but later (**1921**) reported it from Virginia and Bermuda based on two of the collections cited. It is an inconspicuous species; the collections each contain only 2 or 3 small ascocarps. The ascospores are mostly 15-septate. The scales on the stipe are plates of closely adhering hyphae which somewhat resemble the paraphyses of the hymenium. Imai (**1941**) reported the species for Japan. *G. pusillum*, which has been described by Tai (**1944**) from China, appears to be very closely related or the same species.

GEOGLOSSUM PYGMAEUM Gerard ex Durand Ann. Myc. **6**: 429. 1908. (FIGS. 28, 29)

Type: Poughkeepsie, N. Y., W. R. Gerard, in the herbarium of the New York State Museum.

Ascocarps few, clavate, 15–20 mm long, brownish black; ascogenous portion subcylindric to clavate, about ⅓ of the length, 1–2.5 mm thick; stipes slender approximately 0.5 mm thick, squamulose or minutely pubescent; asci, clavate 180–220 × 18–20 μ, 8-spored; ascospores brown, subfusoid or fusoid-clavate, (120–)125–160(–180) × 6–7 μ, mostly 15-septate; paraphyses slightly longer than the asci, hyaline or pale brown below, the terminal cells darker, closely septate above and moderately constricted at the septa, the lower cells cylindric, the upper pyriform, ellipsoid or subgloboid.

Growing on soil.

Specimens studied: New York, W. R. Gerard type (NYS); Tennessee, A. H. Smith *9698* (MICH).

Geoglossum pygmaeum does not differ greatly from *G. pumilum*. The ascospores of *G. pygmaeum* are longer and somewhat more fusoid than those of *G. pumilum*. The ascospores of the type of *G. pygmaeum* are 120–155 μ long and those of the Tennessee specimen are 120–180 μ. The paraphyses of *G. pygmaeum* are more closely septate and more constricted at the septa and the upper cells are darker brown and narrower than those of *G. pumilum*.

The type specimen of *G. pygmaeum* consists of two small ascocarps and the Tennessee specimen consists of three small ascocarps, the five ascomata being all that is known for the species for North America. Patouillard (**1909**) and Luyk (**1919**) state that Zollinger's Pl. Javan. no. *1122* from Java, which was distributed as *Geoglossum hirsutum*, is *Geoglossum pygmaeum*. Patouillard considers it a geographic variety *leveillei*.

GEOGLOSSUM FALLAX Durand, Ann. Myc. **6**: 428. 1908. (FIGS. 30, 31)

Geoglossum subpumilum Imai, Trans. Sapporo Nat. Hist. Soc. **13**: 179. 1934.

Geoglossum proximum Imai, Trans. Sapporo Nat. Hist. Soc. **13**: 180. 1934.

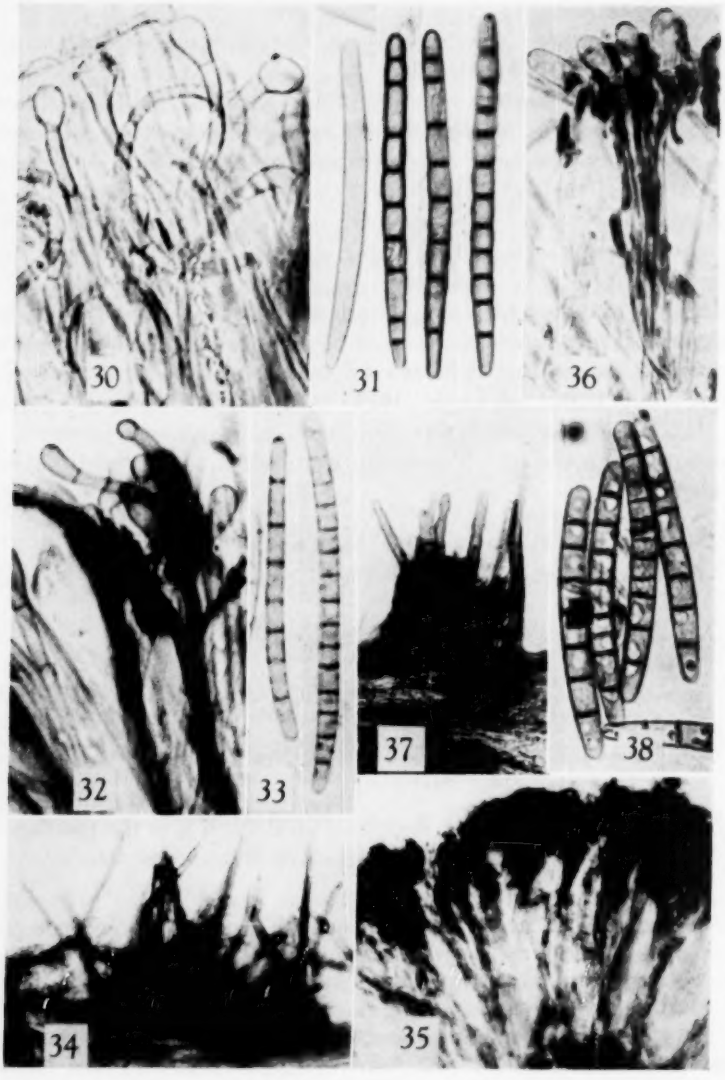
Geoglossum fallax var. *subpumilum* Imai, Journ. Facul. Agr. Hokkaido Imp. Univ. **45**: 215. 1941.

Geoglossum fallax var. *proximum* Imai, Journ. Facul. Agr. Hokkaido Imp. Univ. **45**: 214. 1941.

Type: Ithaca, N. Y., J. D. Reddick (CUP *19197*), in the Herbarium of the Department of Plant Pathology, Cornell University.

Ascocarps scattered to crowded, brown (wood brown to mummy brown when fresh) rarely black, clavate, 1–7 cm long; ascogenous portion compressed, $\frac{1}{3}$ the length, 2–10 mm wide; stipes terete, 1–4 mm thick, squamulose, especially in the upper portion; asci clavate, 150–200 × 18–20 μ; ascospores clavate or clavate cylindric, (45–)66–90(–110) × 5–6 μ, very variable in color and septation, hyaline and brown, 0–13-septate (see discussion), with thin walls; paraphyses hyaline to pale brown, straight or considerably curved above, remotely to moderately septate, not or slightly constricted at septa, with the lower cells cylindric, and the terminal clavate to obovoid, somewhat agglutinating.

On humus and rotting logs. In Michigan this species has been collected from June 12 to Sept. 11.



FIGS. 30, 31. *Geoglossum fallax;* 30, paraphyses; 31, four ascospores 0-, 9-, 8- and 11-septate. *32–35. Geoglossum alveolatum,* type; *32,* paraphyses with adhering amorphous matter; 33, two slightly stained hyaline 7- and 15-septate ascospores; 34, tuft of brown setose hairs on the stipe × 300; 35, portion of hymenium showing epithecium of amorphous matter × 300. 36–38. *Geoglossum intermedium,* type; 36, paraphyses with adhering amorphous matter; *37,* tuft of brown setose hairs on the stipe × 300; 38, four ascospores 7-, 8-, 10-, and 7-septate. × 600 unless otherwise indicated.

Specimens studied: 37 from Michigan (MICH), New Jersey (NY), New York (CUP), Oregon (MICH), Washington (MICH), Bermuda (CUP).

Nannfeldt states that *G. fallax* is one of the commonest species of Scandinavia. It has also been reported by Imai from Japan and China.

As Durand has emphasized, the color of the ascocarps is usually brown, rarely black, differing from most other species. The squamulose condition of the stipes is due to scales which are formed by agglutinated hyphae which appear to be modified paraphyses.

TABLE III

VARIATION IN COLOR AND SEPTATION OF DISCHARGED ASCOSPORES OF GEOGLOSSUM FALLAX EXPRESSED IN PERCENTAGES[2]

Collection[3]	Color and number of septa														
	Hyaline	Brown													
	0	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Smith *30256*	65	9	0	0	1	1	1	3	13	5	2	0	0	0	0
Smith *28323*	31	58	4	0	0	0	0	0	7	0	0	0	0	0	0
Smith *25919*	31	60	0	0	0	0	0	0	0	1	1	1	5	0	1
Smith *31026*	15	74	9	0	1	0	0	0	1	0	0	0	0	0	0
Imshaug *4905*	28	59	0	0	5	0	0	0	2	1	1	4	0	0	0
Simmons *2118*	5	85	1	1	+	0	1	+	2	2	1	1	0	0	0
Type CUP *19197*	8	60	5	2	9	0	+	0	10	2	2	+	2	0	0
CUP *1–171*	2	63	4	1	6	0	0	+	7	2	7	5	3	0	0
Smith *37606*	34	18	5	1	10	2	2	4	11	7	2	2	2	0	0
Smith *31229*	10	65	1	0	10	1	1	3	4	3	1	1	0	0	0
Smith *37183*	35	6	6	1	3	1	3	5	12	5	7	11	5	0	0
Kauffman 1905	3	13	+	0	3	+	4	0	15	20	30	10	1	0	0

The ascospores are very variable in color and septation. Durand states that the spores are "for a long time hyaline" and that they are "at first continuous and multiguttulate, then 3-, finally 7–12-septate." Both hyaline and brown spores are discharged. The hyaline spores are often so abundant that the ascocarps are white-pulverulent from them. The hyaline spores are always non-septate. The brown spores vary from light brown to dark brown and from 0–13-septate. There is a great variation in the proportions of the various kinds of spores in different collections. In a study of 12 collections (TABLE III), the proportions of hyaline spores varied from 2 to 65%. The proportions of non-septate brown spores varied from 6 to 85% and such spores are usually light brown. Among the septate spores there is considerable irregularity. Up to 13-septate were observed in one collection. There is

some evidence of a 1–3–7 sequence in septation but this is obscured by frequent omissions of septa. Beyond the 7-septate condition divisions are even more irregular. It is very doubtful that further development of the ascospores takes place after discharged. These differences are therefore not stages in development but variations in mature spores. While there may be some inherent strain differences it is probable that variations in the proportions of the different kinds of spores are mostly due to conditions affecting the development, or by the age of the ascocarp. Imai (**1941**) gives three varieties of *G. fallax* which he previously had recognized as species. These are distinguished on color and adherence of paraphyses and whether ascospores color slowly or rapidly. In North America it does not seem feasible to recognize forms or varieties.

GEOGLOSSUM ALVEOLATUM (Rehm) Durand, Ann. Myc. **6**: 432. 1908. (FIGS. 32–35)

Leptoglossum alveolatum Rehm, Ann. Myc. **2**: 32. 1904.

Type: Canandaigua, N. Y., E. J. Durand, in the Herbarium of Rehm, Naturhistoriska Riksmuseum Stockholm according to Nannfeldt. Isotype (CUP *1686*) in Herbarium of the Department of Plant Pathology, Cornell University.

Ascocarps solitary to few, scattered, black, clavate, 1.5–5 cm long; ascogenous portion $\frac{1}{3}$ to $\frac{1}{2}$ of the length, 3–4 mm thick, smooth to alveolate, frequently white-pulverulent from discharged ascospores; stipes terete, 1–2 mm thick, with tufted setose hairs; asci clavate, 150–170 × 15 μ, 8-spored; ascospores subcylindric to fusoid cylindric, (50–)67–82(–95) × 4–5 μ, usually hyaline, rarely pale to light brown, 0–15-septate, thin-walled; paraphyses hyaline to brown, straight or slightly curved above, remotely septate, with the lower cells cylindric and the terminal clavate to elongated pyriform, agglutinated at the apices with abundant dark amorphous matter and forming a conspicuous epithecium.

Most commonly on rotting wood, occasionally on soil. In Michigan, this species has been collected from July 23 to September 23.

Michigan, H. A. Imshaug *3640, 3950* (MICH); E. B. Mains *33–528, 33–530* (MICH); E. G. Simmons *2401* (MICH); A. H. Smith *32761, 34163* in part, *37372* (MICH). New York, E. J. Durand, type (CUP *1686*); H. H. Whetzel (CUP *11857*).

This species has also been reported from Japan by Imai.

The paraphyses of this species are regularly agglutinated by dark amorphous matter forming a conspicuous epithecium (FIG. 35). The stipes are tufted setose, due to clumps of stiff dark brown hairs (FIG.

34). Occasionally such hairs occur among the asci in the hymenium. This setose condition of the stipe is not usual in species of *Geoglossum*.

This is an anomalous species of *Geoglossum*, since it produces few colored spores. It was described by Rehm as a species of *Leptoglossum* from a specimen sent to him by Durand under the provisional name *Mitrula alveolata*. Durand later discovered brown spores and transferred it to *Geoglossum*, making the statement that the ascospores are "for a long time hyaline but ultimately becoming pale brown or fuliginous." In this study no brown spores have been seen among the discharged spores, which were all hyaline. They have been noted in a few asci in three collections, CUP *1866*, Mains *33–528* and Smith *32761*. It therefore appears that the mature spores rarely become brown. A study of six of the collections (TABLE IV) shows considerable variation (0–14 or 15) in the septation of the discharged hyaline spores.

TABLE IV

VARIATION IN SEPTATION OF DISCHARGED HYALINE ASCOSPORES OF GEOGLOSSUM ALVEOLATUM EXPRESSED IN PERCENTAGES[2]

Collection[3]	Number of septa															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mains *33–528*	0	0	0	3	1	3	6	24	11	11	10	10	7	11	3	0
Smith *37372*	0	0	3	35	3	3	4	41	3	2	3	2	0	2	1	0
Mains *33–530*	0	0	0	0	0	3	3	19	13	12	10	13	12	7	6	2
Simmons *2401*	+	+	0	11	1	9	3	46	8	9	4	3	3	4	3	3
Imshaug *3950*	0	0	0	3	0	1	3	31	8	5	12	11	9	8	6	3
Imshaug *3640*	0	0	0	2	0	0	3	6	3	7	7	10	9	12	18	25

The septation of the brown spores appears to have a similar variation as far as could be determined from the few seen. There appears to be some evidence that the basic sequence of septation is 0–3–7–15 with other numbers as irregularities.

GEOGLOSSUM INTERMEDIUM Durand, Ann. Myc. **6**: 431. 1908. (FIGS. 36–38)

Type: Knoxboro, N. Y., H. S. Jackson (CUP *335*), in the Herbarium of the Department of Plant Pathology, Cornell University.

Ascocarps black, clavate, 2.0–4.5 cm long; ascogenous portion $\frac{1}{3}$–$\frac{1}{2}$ the length, compressed, 2–6 mm wide, smooth to alveolate; stipes terete, 1 mm thick, with tufted setose hairs; asci clavate, 175–195 × 16–18 μ, 8-spored; ascospores subcylindric, straight to slightly curved, (40–)50–65(–75) × 6 μ, hyaline to very pale brown, the hyaline spores 0–7-

septate, mostly 3-septate, the brown spores 3–11-septate, mostly 7-septate; paraphyses hyaline or light brown, straight, sparsely septate, with lower cells cylindric and terminal pyriform, agglutinated above by abundant dark amorphous matter.

On moist soil.

Specimens studied: New York, H. S. Jackson, type (CUP *335*). Tennessee, E. G. Simmons *2407* (MICH). Virginia, W. H. Long (CUP *9646*, CUP *9647*, CUP *9648*). Ontario, J. H. Faull (CUP *392*).

Geoglossum intermedium is closely related to *G. alveolatum*. It has similar tufted setose hairs on the stipes, paraphyses agglutinated by abundant dark amorphous matter and septate hyaline spores. The ascospores are shorter and broader than those of *G. alveolatum*. Although not abundant, brown-spored asci were found more frequently than in *G. alveolatum*. Both hyaline and brown spores are very variable in septation.

Nannfeldt has described a species *G. elongatum* which appears to be closely related to *G. intermedium*. He states that the stipes have tufts of hairs similar to *G. alveolatum*. The ascospores are given as (40–)50–60 × 5–7 μ, for a long time hyaline, finally pale brown, 0–7-septate. The paraphyses are described as having very irregular and contorted apices and cohering to form a brown apithecium. Amorphous matter is not mentioned.

TRICHOGLOSSUM Boud. Bull. Soc. Myc. France **1**: 110. 1885

Ascocarps clavate to capitate, black or brownish black, fleshy, erect, dry or somewhat viscid, the hymenium covering the upper expanded portion of the ascocarp, hirsute from the setae; stipes slender, terete, hirsute from the setae; asci clavate, inoperculate, 8- or 4-spored or occasionally 2-spored; ascospores clavate, subcylindric or fusoid, brown, usually septate; paraphyses intermixed with the asci, cylindric, usually somewhat enlarged at the apices, erect or usually curved above; setae occurring both in the hymenium and on the stipe, acuminate, dark brown.

Type: *Trichoglossum hirsutum* (Pers. ex Fr.) Boud.

Trichoglossum differs from *Geoglossum* in having abundant setae in the hymenium and on the stipe. There is less variation in the paraphyses than in *Geoglossum*. While most of the species of *Trichoglossum* have 8-spored asci, the 4-spored condition is more common than in *Geoglossum*. *T. velutipes*, which is fairly abundant and widespread in North America, is regularly 4-spored. *T. tetrasporum*, which is known

in North America only from the type collection, differs from *T. hirsutum* only in being 4-spored. In *T. rasum* (*T. wrightii*) 4-spored asci occur in some collections along with the 8-spored asci.

The ascospores of North American species of *Trichoglossum* are brown when discharged. According to Tai (**1944**) *T. durandii* Teng in China has ascospores which remain "hyaline for a long time, finally becoming brownish" which may indicate that both hyaline and brown spores are produced as in *Geoglossum alveolatum*. However, Teng (**1932**) described the spores as hyaline. Imai (**1942**) has proposed a new genus *Leucoglossum* for it.

There is much greater variation in the septation of the ascospores in *Trichoglossum* than in *Geoglossum*. As has already been discussed there appear to be two different methods of septation. The species are basically 7- or 15-septate or they may be very variable in septation, possibly basically 5- or 11-septate. However, deviations from the basic conditions are frequent and varied. In *T. rasum* (*T. wrightii*) there is not only variation in number of spores in the ascus and in septation but asci with very abnormal ascospores are not unusual, indicating considerable genetic instability.

Trichoglossum hirsutum and *T. velutipes* occur in both western and eastern North America. *T. rasum* is entirely tropical. The other species appear to be limited to eastern North America or with a few records from the tropics. More than half of the collections studied of *Trichoglossum* were *T. hirsutum*. The other species in order of prevalence are *T. velutipes*, *T. farlowii*, *T. walteri*, *T. variabile*, *T. octopartitum* and *T. rasum*. *T. confusum* and *T. tetrasporum* are known for North America only from the type specimens.

KEY TO SPECIES OF TRICHOGLOSSUM

1. Ascospores 0–5-septate, rarely more, commonly 3-septate.....*T. farlowii*
1. Ascospores mostly more than 5-septate.....2
2. Ascospores 7-septate, rarely more.....3
2. Ascospores more than 7-septate or, if variable in septation, many more than 7-septate.....5
3. Ascospores fusoid or fusoid-clavate, mostly more than 100 μ long..*T. octopartitum*
3. Ascospores subcylindric or clavate-cylindric, mostly less than 100 μ long.....4
4. Ascospores (60–)72–100(–125) μ long.....*T. walteri*
4. Ascospores (45–)55–65(–75) μ long.....*T. confusum*
5. Ascospores 15-septate or, if variable in septation, many 15-septate.....6
5. Ascospores very variable in septation, few or none 15-septate.....7
6. Asci 8-spored.....*T. hirsutum*
6. Asci 4-spored.....*T. tetrasporum*

7. Asci 4-spored.....*T. velutipes*
7. Asci 8-spored or mostly 8-spored.....8
8. Ascospores mostly 6–9-septate.....*T. rasum*
8. Ascospores mostly 10–13-septate.....*T. variable*

TRICHOGLOSSUM FARLOWII (Cooke) Durand, Ann. Myc. **6**: 438. 1908. (FIG. 39)

Geoglossum farlowii Cooke, Grevillea **11**: 107. 1883.

Type: Newton, Mass., W. G. Farlow, in the Kew Herbarium according to Durand.

Ascocarps scattered or crowded, black or brownish black, clavate, 3–8 cm long; ascogenous portion $\frac{1}{3}$ to $\frac{1}{2}$ of the length, compressed, 3–15 mm wide, hirsute from the setae; stipes terete, 1.5–4.0 mm thick, more or less densely hirsute from the setae; asci clavate, 150–180 × 15–20 μ, 8-spored; ascospores subcylindric to clavate-cylindric, (45–)57–75(–90) × 5–7 μ, 0–5-septate, very rarely more, usually mostly 3-septate, light brown; paraphyses cylindric, somewhat enlarged at the apices, moderately septate, curved to circinate above; setae abundant, dark brown, acuminate.

On moist to wet soil occasionally in moss and on rotten logs. In Michigan this species has been collected from August 8 to October 7.

Specimens studied: 54 from Alabama (MICH), Florida (NY), Massachusetts (NY), Michigan (MICH), Mississippi (NY), New Hampshire (NY), New Jersey (NY, NYS), New York (UC, MICH, NY), North Carolina (T), Ohio (MICH, NY), Pennsylvania (MICH, NY, UC), Tennessee (MICH, T), Vermont (MICH), Virginia (NY, T, UC), Nova Scotia (MICH), Ontario (NY).

Trichoglossum farlowii has also been reported from Japan by Imai.

Trichoglossum farlowii is distinguished from other species by its short, almost cylindric ascospores which are 0–5-septate, or rarely 6- or 7-septate. TABLE V gives the results of a study of the variation in the septation of the discharged ascospores of 10 collections. In all except one, 3-septate spores are by far the most abundant. In one collection non-septate spores predominate. The septation appears to follow the 0–1–3–5 sequence. The two terminal cells in three-septate spores are longer than the two medial cells (FIG. 39). Measurements of thirty-nine 3-septate spores gave an average of 20.5 μ for the terminal cells and 13.9 μ for the medial. Measurements of nine 5-septate spores gave the average length of the medial cells as 13.8 μ and of the four others (two at each end) as 10.6 μ. This supports Nannfeldt's suggestion that the second divisions of the ascospores in this species are unequal and that then the two long end cells divide. Division rarely continues beyond the 5-septate condition and then one, or more rarely, two cells divide.

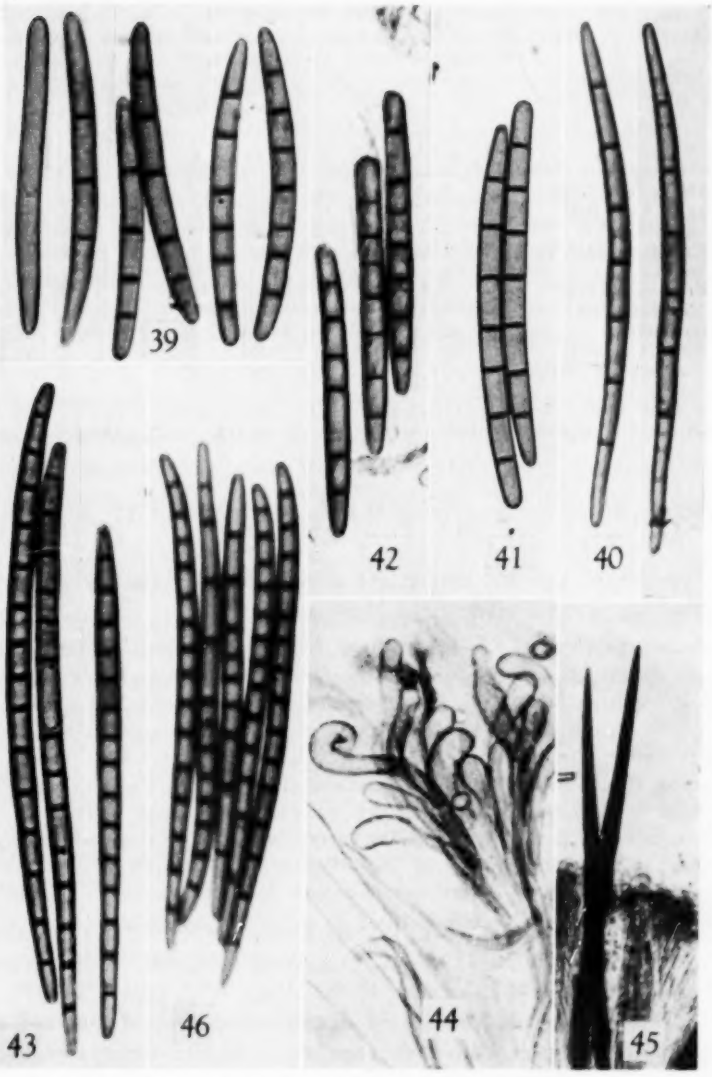


FIG. 39. *Trichoglossum farlowii*, six ascospores 0-, 3-, 4-, 5-, 6- and 7-septate. 40. *Trichoglossum octopartitum*, two 7-septate ascospores. 41. *Trichoglossum walteri*, two 7-septate ascospores. 42. *Trichoglossum confusum*, type, one 6-septate and two 7-septate ascospores. 43–45. *Trichoglossum hirsutum* var. *hirsutum*; 43, one 14-septate and two 15-septate ascospores; 44, paraphyses; 45, setae of the hymenium × 300. 46. *Trichoglossum hirsutum* var. *irregulare*, type, five ascospores 15-, 12-, 9-, 13- and 15-septate. × 600 unless otherwise indicated.

TABLE V

VARIATION IN THE SEPTATION OF DISCHARGED ASCOSPORES OF TRICHOGLOSSUM FARLOWII EXPRESSED AS PERCENTAGES

Collection[a]	Number of septa							
	0	1	2	3	4	5	6	7
Mains Sept. 1935	3	17	6	73	0	2	0	0
Smith Oct. 1935	1	2	6	84	0	7	0	0
Smith *35975*	0	8	0	90	2	0	0	0
Kauffman Sept. 1924	1	1	3	68	7	13	5	2
Smith *7703*	3	19	4	67	4	3	0	0
Edgecombe 1938	2	5	5	83	3	2	0	0
Mains *32–670*	0	6	7	63	3	19	2	0
Smith *9995*	80	13	0	3	2	2	0	0
Ray UC *554048*	16	15	11	58	2	7	0	0
Connors UC *569326*	1	3	3	84	2	7	0	0

Two- and 4-septate spores are the result of irregular failures of cells to divide.

TRICHOGLOSSUM OCTOPARTITUM Mains, Am. Jour. Bot. **27**: 325. 1940. (FIG. 40)

Type: San Agustin, British Honduras, E. B. Mains *4097,* in the Herbarium of the University of Michigan.

Ascocarps clavate, 1.5–4.0 cm long, black; ascogenous portion $\frac{1}{4}$ to $\frac{1}{3}$ of the length, 2–4 mm wide, hirsute from setae; stipes terete, slender, 1.0–1.5 mm thick, hirsute from setae; asci clavate, 175–200 × 18–20 μ, 8-spored; ascospores fusoid to fusoid clavate, attenuated to both ends from above the middle, (80–)100–120(–150) × 4.0–5.5 μ, brown, 7-septate, rarely less; paraphyses somewhat exceeding the asci, sparsely septate, cylindric with terminal cell somewhat enlarged, curved to circinate above; setae dark brown, acuminate, projecting considerably beyond the hymenium.

On soil.

Specimens studied: Ohio, W. B. Cooke *941* (NY). Tennessee, Hesler and Smith *10742* (MICH). West Virginia, L. W. Nuttall *577* (NY). British Honduras, E. B. Mains *4097,* type (MICH).

Trichoglossum octopartitum is distinguished from *T. walteri* and *T. confusum* which also have regularly 7-septate ascospores by its fusoid to fusoid-clavate, longer ascospores.

TRICHOGLOSSUM WALTERI (Berk.) Durand, Ann. Myc. **6**: 440. 1908. (FIG. 41)

Geoglossum walteri Berk. Hedwigia **14**: 39. 1875.

Geoglossum rehmianum P. Henn. Hedwigia **38**: (80). 1900.

Trichoglossum rehmianum (P. Henn.) Durand, Ann. Myc. **6**: 439. 1908.

Type: Appolo Bay, Australia, Walter, in Kew Herbarium according to Durand.

Ascocarps scattered to crowded, black to brownish black, clavate, 3–10 cm long, hirsute from setae; ascogenous portion $\frac{1}{3}$ to $\frac{1}{2}$ of the length, often compressed, 4–10 mm wide; stipes terete, 2–6 mm thick, hirsute from setae; asci clavate, 165–200 × 15–18 μ, 8-spored; ascospores subcylindric, narrowing below, (60–)72–100(–125) × 5–6 μ, light brown, mostly 7-septate; paraphyses light brown, cylindric, moderately septate, curved to circinate at the apices; setae abundant, dark brown, acuminate.

On soil and rotting wood. In Michigan this species has been collected from August 3 to October 8.

Specimens studied: 32 from Connecticut (UC, NY), Indiana (NY), Massachusetts (MICH, UC, NY), Michigan (MICH), New Hampshire (MICH, NY), New Jersey (NY), New York (MICH, NYS, NY), North Carolina (T), Pennsylvania (MICH, NY), Tennessee (MICH), Virginia (MICH, NY), Jamaica.

T. walteri has also been reported from Brazil, Europe, Australia and eastern Asia.

T. walteri has more cylindric, shorter and somewhat wider ascospores than *T. octopartitum*. It is the most common species having regularly 7-septate ascospores. Tai (**1944**) has described *T. kunmingense* from China with ascospores clavate-cylindric to subcylindric and 104–144 μ long. He states that it differs from *T. walteri* mainly in longer ascospores. It appears to be intermediate between *T. walteri* and *T. octopartitum* although the ascospores are not as regularly septate as either. *T. walteri*, *T. octopartitum*, *T. kunmingense* and *T. confusum* are closely related and with the accumulation of more collections it may be desirable to unite them in one species.

TRICHOGLOSSUM CONFUSUM Durand, Mycologia **13**: 185. 1921. (FIG. 42)

Type: Blowing Rock, North Carolina, E. J. Durand no. *1934*, in the Herbarium of the Department of Plant Pathology, Cornell University.

Ascocarps black, clavate, 1.5–2.5 cm long, hirsute from setae; ascogenous portion $\frac{1}{3}$ to $\frac{1}{2}$ of the length, furrowed or compressed; stipe terete,

1–1.5 mm thick, hirsute from setae; asci narrowly clavate, 150–200 × 12–16 μ, 8-spored; ascospores subcylindric (45–)55–66(–75) × 5–6 μ, 3–7-septate, mostly 7-septate; paraphyses pale brown, cylindric, slightly increasing in width upward, straight or curved above, projecting beyond the asci; setae abundant, dark brown, acuminate.

On soil.

North Carolina: E. J. Durand, *1934*, type (CUP).

This species was treated by Durand in his monograph under the name *T. rehmianum*. Later Durand (**1921**) concluded that *T. rehmianum* was the same as *T. walteri* and a new species was described. Very few discharged spores have been seen. In crushed mounts there is a variation in the septation of the ascospore which may be due in part to differences in maturity. It differs from *T. walteri* in having somewhat shorter ascospores with more variable septation. It apparently is known for North America only from the type. Teng (**1934**) has reported one collection from China.

TRICHOGLOSSUM HIRSUTUM (Pers. ex Fr.) Boud. Bull. Soc. Myc. France **1**: 110. 1885.

Geoglossum hirsutum Pers. ex Fr. Syst. Myc. **1**: 488. 1821.

Trichoglossum gracile Pat. Bull. Soc. Myc. France **25**: 131. 1909.

Lectotype: In Persoon Herbarium, Rijks Herbarium, Leiden, according to Durand.

Ascocarps scattered to gregarious, black or brownish black, clavate to capitate, 1–8 cm long; ascogenous portion compressed, up to 2 cm long, 2–5 mm wide, hirsute from setae; stipes slender, 2–3 mm thick, hirsute from setae; asci clavate, 180–275 × 18–25 μ, 8-spored; ascospores fusoid-clavate, narrowing toward each end from above the middle, 80–170 × 5–7 μ in var. *hirsutum*, longer in other varieties, in some varieties 15-septate, in others septation very variable but with many spores 15-septate; paraphyses brown, cylindric, moderately septate, somewhat enlarged and straight or curved to circinate above; setae abundant, acuminate, dark brown, projecting ¼ to ⅓ their lengths above the hymenium.

A. TRICHOGLOSSUM HIRSUTUM var. **hirsutum.** (FIGS. 43–45)

Ascospores clavate, (80–)110–140(–170) × 5–7 μ, mostly 15-septate.

On rotting wood, on soil and in sphagnum. In Michigan this variety has been collected from July 18 to October 17.

Specimens studied: 130 from California (UC, NY, MICH), Connecticut (UC), Idaho (MICH), Massachusetts (MICH, NY), Michi-

gan (MICH), New Hampshire (MICH, UC), New Jersey (NY), New York (NY, NYS, MICH), North Carolina (MICH, T, NY), Ohio (MICH), Oregon (MICH, NY), Pennsylvania (MICH), Rhode Island (NY), Tennessee (T, MICH), Virginia (NY), Washington (MICH), West Virginia (MICH), Bermuda (NY), Jamaica (NY), Ontario (MICH, NY, CU, T), Quebec (MICH).

B. TRICHOGLOSSUM HIRSUTUM var. **longisporum** (Tai) comb. nov. (FIG. 47)

Trichoglossum longisporum Tai, Lloydia **7**: 156. 1944.

Asci clavate, 225–275 × 20–22 μ; ascospores fusoid-clavate, (120–) 133–180(–195) × 6–7 μ, mostly 15-septate.

Type: Chichushan, Yunnan, China, Sept. 18, 1938, C. C. Cheo *5089*.

Specimens studied: California, A. H. Smith *3796, 9001, 9375, 9412, 9707* (MICH).

Tai describes the asci of *T. longisporum* as 237–281 × 19–25 μ and the ascospores 156–190 × 6–7 μ.

C. TRICHOGLOSSUM HIRSUTUM var. **irregulare** var. nov. (FIG. 46)

Var. *hirsuto* simile sed ascosporis 0–15-septatis.

Type: Lower Tahoma, Mt. Ranier Nat. Park, Washington, Sept. 9, 1948, D. E. Stuntz, A.H.S. *30977* (MICH).

Specimens studied: California, A. H. Smith *9413, 9453, 9706* (MICH); Silva and Neeley (CU *585268*); N. L. Gardner (CU *513555*). Michigan, A. H. Smith *33043, 38307* (MICH); H. A. Imshaug *3897* (MICH). New Hampshire, E. B. Mains *4218* (MICH). Oregon, A. H. Smith *7995, 18216, 18223, 18822* (MICH). Tennessee, E. Simmons *2408, 2409* (MICH); A. H. Smith *10492* (MICH). Washington, A. H. Smith *17938, 31079* (MICH); D. E. Stuntz, A.H.S. *30977*, A.H.S. *30128* (MICH); E. Simmons (MICH). Ontario, A. H. Smith *26431* (MICH).

TABLE VI gives the results of a study of eleven collections of variety *irregulare* for variation in septation. The percentages of 15-septate spores vary considerably (*22–82%*) in the collections and are in all instances more than any other class. The variety is basically 15-septate and the deviations are mostly due to irregular failures of one or more septa to develop as is evident in FIG. 46. Rarely a 16- or 17-septate spore is produced. The ascospores of this variety are (90–)100–150(–165) × 5–7 μ.

TABLE VI

VARIATION IN THE SEPTATION OF DISCHARGED ASCOSPORES OF TRICHOGLOSSUM HIRSUTUM VAR. IRREGULARE EXPRESSED AS PERCENTAGES

Collection[a]	Number of septa															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Smith *10492*	0	0	0	0	0	0	0	0	0	0	2	2	1	3	10	82
Smith *18216*	0	0	0	0	0	0	0	0	0	0	1	1	4	4	13	77
Smith *26431*	0	0	0	0	1	0	0	1	1	1	2	2	4	8	17	63
Smith *31079*	0	0	1	0	0	0	0	1	1	3	3	4	3	10	13	61
Smith *7995*	0	0	0	0	0	0	0	0	1	2	2	3	3	15	14	60
Smith *30977*	0	0	0	0	0	0	0	2	1	3	3	3	5	12	19	52
Smith *18822*	0	0	0	0	0	0	0	2	5	3	2	6	4	14	20	44
CU *513555*	0	0	0	0	0	2	1	8	6	6	7	9	6	7	6	42
Smith *31028*	0	0	0	0	0	0	0	9	6	1	3	6	6	14	17	38
Smith *18223*	0	0	0	0	0	0	1	4	2	3	4	8	12	12	17	37
Smith *38307*	6	4	3	3	4	4	1	5	3	5	5	8	8	7	12	22

D. TRICHOGLOSSUM HIRSUTUM var. **heterosporum** var. nov. (FIG. 48)

Var. *hirsuto* simile sed ascosporis 8–21-septatis.

Type: Husky Gap Trail, Great Smoky Mts. Nat. Park, Tennessee, Aug. 4, 1938, A. H. Smith *9348* (MICH).

The asci of this form are clavate, 175–200 × 17–20 μ. The ascospores are fusoid-clavate, narrowing to each end from above the middle, (95–) 120–150(–160) × 5–6 μ. A study of the variation of septation of 362 discharged spores gave the following: 1%, 8-septate; 1%, 9-septate; 1%, 10-septate; 16%, 11-septate; 15%, 12-septate; 15%, 13-septate; 15%, 14-septate; 15%, 15-septate; 10%, 16-septate; 4%, 17-septate; 3%, 18-septate; 2%, 19-septate; 1%, 20-septate; 1%, 21-septate. This variety, therefore, is considerably more variable in septation than var. *irregulare*. It has 64% of the spores less than 15-septate and 21% more. It appears, however, to be a *T. hirsutum* in which irregular deviations from the 15-septate condition are produced by failures of septa to form and by continued irregular septations beyond the 15-septate stage as is indicated by the variations in size of the cells.

E. TRICHOGLOSSUM HIRSUTUM var. **multiseptatum** var. nov. (FIG. 49)

Var. *hirsuto* simile sed ascosporis (145–)160–195(–210) × 6 μ, 12–22-septatis.

Type: On soil near Calderwood, Blount Co., Tennessee, Aug. 21, 1932, L. R. Hesler, T *14156* (MICH).

Specimens studied: Tennessee, L. R. Hesler, T *5479*, T *14156* (MICH).

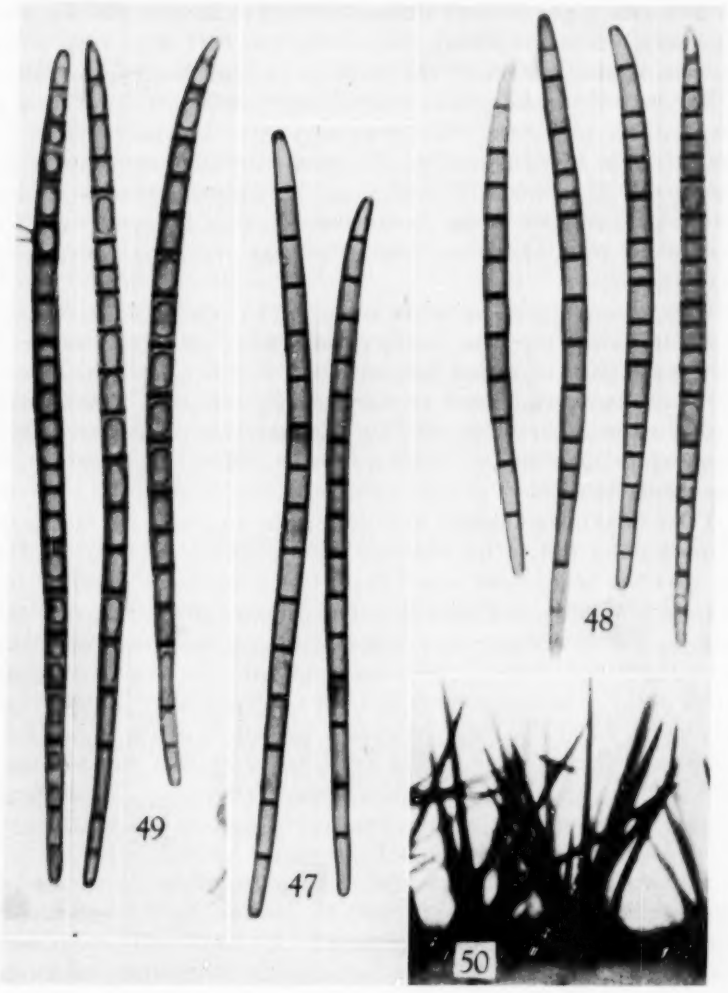


FIG. 47. *Trichoglossum hirsutum* var. *longisporum*, two 15-septate ascospores. 48. *Trichoglossum hirsutum* var. *heteorsporum*, type, four ascospores 11-, 15-, 17- and 21-septate. 49. *Trichoglossum hirsutum* var. *multiseptatum*, type, three ascospores 21-, 19- and 16-septate. 50. *Trichoglossum variabile*, setae of the stipe × 300. × 600 unless otherwise indicated.

This form has slender-clavate ascocarps, 2.5–4.0 cm long and 1.5–4.0 mm wide. The asci are clavate, 210–225 × 20–25 μ and 8-spored. The ascospores are fusoid-clavate, (145–)160–195(–210) × 6 μ. The septation is very variable. The variation as determined from a study of 500 discharged ascospores of collections T *5479* and T *14156* is as follows: 1%, 13-septate; 4%, 14-septate; 16%, 15-septate; 22%, 16-septate; 27%, 17-septate; 17%, 18-septate; 10%, 19-septate; 3%, 20-septate. A 12-septate, a 21-septate and a 22-septate spore were seen. In this form only 5% of the spores were less than 15-septate and 79% were more than 15-septate, with 17-septate spores occurring most abundantly.

There is considerable variation in the size and shape of the ascocarps of *T. hirsutum*. They are usually more or less clavate but occasionally they are capitate. Durand has suggested that *Geoglossum capitatum* Pers. may have been based on the capitate condition. This has been recognized as a variety or form by various authors (see Imai **1941**). *Geoglossum hirsutum* var. *leotioides* Cooke (**1879**) also appears to be a capitate condition.

There is also considerable variation in the length of the ascospores. Durand states that in the lectotype the ascospores are 98–150 μ long, the majority being more than 120 μ. In the summary given by Imai of the size of ascospores given by various authors 160 μ is the maximum length. Tai has recognized a species *T. longisporum* in China which is distinguished from *T. hirsutum* principally by longer ascospores (156–190 μ). Several collections from California were found to have ascospores 120–195 μ long. Although the length of the ascospores is more variable than given by Tai for *T. longisporum* it seems questionable that they should be placed in a separate category. The distinction from *T. hirsutum* then becomes less distinct and the taxon is here considered a long-spored variety of *T. hirsutum*.

T. hirsutum is usually described as having regularly 15-septate ascospores. This regularity is stressed by Durand for the typical form. He states that in the Persoon lectotype "one occasionally meets with a spore with less than 15 septa but these are manifestly abnormal." Durand recognized two forms *variabile* and *wrightii* which differed in having variable septation of the ascospores, which were very rarely 15-septate. Durand later recognized f. *wrightii* as a species and Nannfeldt has raised f. *variabile* to specific rank. As discussed under these species the septation appears to be on a different basis than in *T. hirsutum*.

Most of the collections in this study were found to be fairly regularly

15-septate with only a few spores less, usually 13- or 14-septate. However in some collections there was a considerable departure from this condition. Some had ascospores of the typical length and others had longer. It has seemed best to recognize these deviations as varieties. Variety *irregulare* has ascospores similar in length to f. *hirsutum*. While 15-septate spores are more abundant than any other class, 18 to 78% of the spores have septations less than 15, varying from 0–14, and rarely having 16- and 17-septate spores. The amount of variation differs considerably among the collections as shown in TABLE VI and there is gradation into var. *hirsutum*. Var. *heterosporum* has ascospores similar in length to var. *hirsutum* and var. *irregulare*. They are more variable in septation than the latter. In part of the spores septation has continued beyond the 15-septate condition. Var. *multiseptatum* has ascospores which are as long as var. *longisporum*. It differs from that variety in having variable septation of the ascospores, which are mostly more than 15-septate. Tai (**1944**) has described a species *T. persoonii* with ascospores 162–200 × 5–6 μ and 13–20-septate, mostly 15–19-septate. The spores are described as clavate-cylindric but illustrated as fusoid with acute ends.

TRICHOGLOSSUM TETRASPORUM Sinden & Fitzp. Mycologia **22**: 60. 1930. (FIG. 52)

Type: Labrador Lake, New York, H. M. Fitzpatrick, 1924 (CUP *17779*) in the herbarium of the Department of Plant Pathology, Cornell University.

Ascocarps black, clavate, 3–8 cm long; ascogenous portion approximately $\frac{1}{3}$ of the length, compressed, hirsute from the setae; stipes terete, slender, 1–2 mm thick, hirsute from the setae; asci clavate, 175–200 × 20–25 μ, 4-spored or occasionally with fewer spores; ascospores clavate or fusoid-clavate, narrowing toward each end from above the middle, (110–)125–145(–150) × 6–7 μ; dark brown, 0–17-septate, mostly 15-septate; paraphyses brown, cylindric, somewhat enlarged and curved to circinate at the apices, moderately septate; setae dark brown, acuminate, projecting slightly above the hymenium.

In North America *T. tetrasporum* is known only from the type collection, which was loaned for this study. Since the specimen had been preserved in liquid it was not possible to make a satisfactory study of discharged ascospores. In mounts of the hymenium the ascospores varied from 0 to 17 in septation with 15-septate spores the most abundant. Sinden and Fitzpatrick in their discussion of the species state that the young ascus contains the fundaments of 8 spores of which only 4 develop. In some asci it was noted that of the four only 2 became

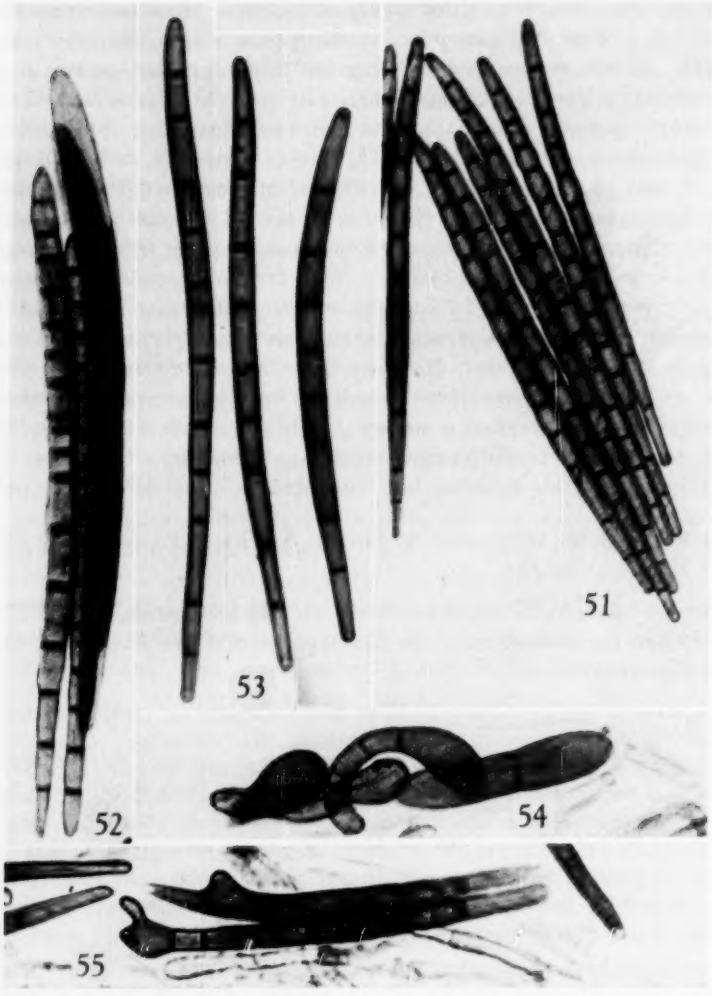


FIG. 51. *Trichoglossum variabile*, ascospores with 10, 12, 13, 14 and 15 septa. 52. *Trichoglossum tetrasporum*, type, four spores from an ascus, two septate and two non-septate. 53. *Trichoglossum velutipes*, three ascospores 11-, 9-, and 8-septate. 54, 55. *Trichoglossum rasum* (*T. wrightii*, type); 54, abnormal ascospores of a 4-spored ascus; 55, abnormal ascospores. All × 600.

septate and the other two remained non-septate and usually much lighter brown (FIG. 52).

The species has also been reported from Great Britain and China. Nannfeldt states that one of three ascocarps of the Uppsala copy of Cooke's Fungi Brit. ed. II no. *393* was found to be *T. tetrasporum* and the other two were *T. hirsutum* and *Geoglossum glutinosum*. Tai (**1944**) has described a variety *brevisporum* based on one collection from Yunnan, China, which was found to have ascospores 117–137 × 6–7 μ.

Tai has described a 4-spored species *T. yunnanense* which he states is close to *T. longisporum* and which differs from *T. tetrasporum* in having longer and narrower asci, longer ascospores and by the shape of the ascospores. The part of the type (*T. tetrasporum* no. *27985*) in the herbarium of the Department of Plant Pathology of Cornell University has been studied. The asci are 200–290 × 19–22 μ. The ascospores are (125–)140–190 × 6–7 μ and fusoid-clavate. The spores have 10–25 septa with approximately 60% 15-septate. *T. yunnanense* appears to be a longer, more variable, septate-spored variant of *T. tetrasporum* for which the name *T. tetrasporum* var. **yunnanense** (Tai) comb. nov. is proposed.

T. tetrasporum differs from *T. hirsutum* mostly in having 4-spored instead of 8-spored asci and it has similar variations. Unlike *T. hirsutum*, which is both common and widespread, *T. tetrasporum* is known only from 4 collections which are from widely separated localities. This suggests independent originations from *T. hirsutum* which nowhere appear to have been able to develop and maintain themselves.

TRICHOGLOSSUM VELUTIPES (Peck) Durand, Ann. Myc. **6**: 434. 1908. (FIG. 53)

Geoglossum hirsutum var. *americanum* Cooke, Mycographia **1**: 3. 1875.

Geoglossum velutipes Peck, Rep. N. Y. State Museum **28**: 65. 1876.

Geoglossum americanum (Cooke) Sacc. Syll. Fung. **8**: 46. 1889.

Lectotype: H. A. Warne, Oneida, Madison Co., New York, in the Herbarium of the New York State Museum.

Ascocarps scattered to crowded, black or brownish black, clavate, 2–5 cm long; ascogenous portion ⅛–¼ the length, compressed, 2–12 mm wide, hirsute from the setae; stipes slender, terete, 1.5–2.5 mm thick, hirsute from the setae; asci clavate, 180–200 × 16–20 μ, 4-spored; ascospores clavate, (90–)110–145(–160) × 6–7 μ, (0–)7–11(–13)-septate, mostly 9-septate (see TABLE VII), paraphyses brown, moderately septate, cylindric, slightly enlarged above, curved to circinate; setae dark

brown, acuminate, abundant, projecting ⅓ to ½ their lengths above the hymenium.

On soil and rotting wood. In Michigan this species has been collected from July 25 to September 11.

Specimens studied: 59 from Connecticut (NY, CU), Idaho (MICH), Maryland (MICH), Michigan (MICH), New Hampshire (MICH), New Jersey (MICH), New York (MICH, NY, NYS), Oregon (MICH), Tennessee (T), Washington (MICH), West Virginia (T), Nova Scotia (MICH), Ontario (MICH, NY, CU), Quebec (MICH), Jamaica (NY).

Peck described *Geoglossum velutipes* from two specimens collected at Oneida and Northville, N. Y. In his description he states that the spores are "at first simple or triseptate, then elongated and none to eleven-septate." In his comments he states that the difference between the young and the mature spores is quite noticeable. Durand reports that the Oneida specimen has short 0–3-septate ascospores and is *Trichoglossum farlowii* and the Northville specimen has longer 9–11-septate ascospores and should be recognized as the type of *T. velutipes*. The results of a study of the syntypes from the herbarium of the New York State Museum are not in agreement with Durand's statement. The Northville collection is a mixture, part of the ascocarps having 8-spored asci with ascospores 0–7-septate and being 50–80 μ long (*T. farlowii*) and part having 4-spored asci with ascospores 7–12-septate and 120–170 μ long. The Oneida specimen has 4-spored asci with ascospores 6–13-septate and 125–185 μ long. The specimens in the herbarium of the New York State Museum do not bear annotations by Durand. There is a specimen in the herbarium of the New York Botanical Garden collected by H. A. Warne at Oneida, N. Y., in 1879, which bears the name *Geoglossum velutipes* and is annotated *Trichoglossum farlowii* by Durand. This specimen, which is *T. farlowii*, may possibly be the basis for Durand's statement.

It is evident that, as Durand has suggested, Peck mistook *T. farlowii* as the young immature condition of *T. velutipes*, misled probably by the mixed Northville collection. The name *T. velutipes* should apply to the species having 4-spored asci and long, 9–11-septate ascospores, since Peck considered this the mature condition and since later he so illustrated the asci and ascospores. Since the Northville collection in the herbarium of the New York State Museum is a mixture of *T. velutipes* and *T. farlowii* and the Oneida is entirely *T. velutipes* the latter should be recognized as the lectotype.

Peck apparently continued to hold his original concept, since of five

additional collections in the herbarium of the New York State Museum, probably identified by Peck, three are *Trichoglossum farlowii*, one a mixture of *T. farlowii* and *T. velutipes* and one *T. velutipes*.

There is considerable variation in the septation of the ascospores in this species. TABLE VII gives the results of a study of 10 collections for variation in septation. The over-all variation was 0–13. However, most of the spores showed septations between 7 and 11 with 9 being the predominant septation in all but one of the collections. On account of the small number of 11-septate spores it is doubtful if this is basically an 11-septate species as suggested by Sinden and Fitzpatrick (**1930**). The septation is very irregular and the sequence is not clear.

TABLE VII

VARIATION IN THE SEPTATION OF DISCHARGED ASCOSPORES OF TRICHOGLOSSUM VELUTIPES EXPRESSED AS PERCENTAGES[2]

Collection[3]	Number of septa													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Mains *33–590*	0	0	0	0	0	0	0	1	20	75	4	0	0	0
Mains *33–495*	0	0	0	0	0	+	+	2	3	41	25	22	3	3
Mains *4230*	0	0	0	0	0	1	1	0	4	47	28	17	2	0
Smith *17381*	0	0	0	0	0	2	4	29	31	33	1	0	0	0
Smith *32908*	0	0	0	0	0	0	1	4	13	75	6	1	0	0
Connors *7344*	0	0	0	0	0	1	0	11	24	56	7	1	0	0
Wehmeyer *1380*	0	0	0	0	0	0	0	1	20	71	7	1	0	0
Lewis *9–29–37*	0	0	0	0	0	0	1	19	27	51	2	0	0	0
Smith *18221*	2	2	1	2	2	6	9	50	17	8	1	0	0	0
Warne lectotype	0	0	0	0	0	0	1	3	1	55	27	12	1	+

T. velutipes is the most common of the 4-spored species. It is found mostly in North America and is apparently known elsewhere from only two collections reported by Tai (**1944**) from China.

TRICHOGLOSSUM VARIABILE (Durand) Nannf. Ark. Bot. **30A**: 64. 1942. (FIGS. 50, 51)

Trichoglossum hirsutum f. *variabile* Durand, Ann. Mycol. **6**: 437. 1908.

Type: Knoxboro, New York, H. S. Jackson, Aug. 20, 1904 (CUP *4–251*) in the herbarium of the Department of Plant Pathology, Cornell University.

Ascocarps dark brown to black, clavate, 2–4 cm long; ascogenous portion compressed, 2–5 mm wide, hirsute from setae; stipes terete, slender, 1 mm thick, hirsute from setae; asci clavate, 150–200 × 18–20 μ, 8-spored; ascospores brown, subfusoid or fusoid-clavate, narrowing to

each end from above the middle, (80–)110–130(–150) × 4.5–6 μ, 4–16-septate, mostly 10–13-septate; paraphyses brown, moderately septate, cylindric below, enlarging and somewhat clavate above, straight or slightly curved above, somewhat agglutinated; setae abundant, dark brown, acuminate, projecting ⅓ beyond the hymenium.

On wet and dry soil and occasionally in sphagnum.

Specimens studied: 13 from Delaware (CUP), New York (CUP, NY), North Carolina (CUP), Tennessee (T), Virginia (CUP), Panama Canal Zone (NY).

T. variabile has also been reported from Sweden, China and Japan.

This species was originally described by Durand as a form of *T. hirsutum* differing in having 8–14-septate ascospores. Nannfeldt has

TABLE VIII

VARIATION IN THE SEPTATION OF DISCHARGED ASCOSPORES OF TRICHOGLOSSUM VARIABLES EXPRESSED IN PERCENTAGES[2]

Collection	Number of septa												
	4	5	6	7	8	9	10	11	12	13	14	15	16
Jackson Type CU *4–251*	0	+	0	1	3	10	15	36	19	8	6	2	0
Jackson CU *4–252*	0	0	0	0	0	5	8	39	36	8	4	0	0
Jackson CU *4–253*	0	0	0	0	0	0	3	15	31	34	12	5	0
Durand CU *4–254*	0	0	0	0	1	2	5	37	22	18	14	1	0
Long CU *4–255*	0	0	0	0	+	+	4	27	34	24	9	2	0
Long CU *4–256*	0	0	0	0	1	1	2	32	35	20	6	2	1
House *13.44* NYS	+	+	1	6	13	20	30	27	1	0	+	0	0
Hesler & Sharp T *7966*	0	0	0	0	0	2	7	32	35	16	5	3	0
Seaver *9–30–1913* NY	0	1	1	1	3	19	18	15	15	25	1	1	0
Long *2296* NY	0	0	0	1	0	1	7	31	35	20	5	0	0
Martin *4072* NY	0	0	0	0	0	3	13	37	31	12	3	1	0

concluded that the method of septation is different from that in *T. hirsutum* and he has therefore recognized it as a separate species. TABLE VIII gives the results of a study of the discharged ascospores of 11 collections, including the type, for variation in septation. The over-all variation in septation was found to be 4–16. As shown, the collections differ somewhat in their variability with the maxima varying from 10–13. Nannfeldt has suggested that *T. variabile* is basically 11-septate and that variation from this is due to one or more septa failing to form, or one or more supernumerary septa being formed. The results of TABLE VIII give some support to this idea. If so the irregular departures from a 0–1–3–5–11 sequence are so many that it has not been possible to reach a conclusion. However, a comparison with TABLE VI shows that the pattern of variation is distinctly different from that shown by the variable forms of *T. hirsutum*, justifying the recognition of a separate species.

T. variabile differs from *T. velutipes* not only in having 8-spored asci instead of 4-spored but also in the variation in septation of the ascospores, which is mostly 10–13 instead of mostly 7–11.

TRICHOGLOSSUM RASUM Pat. Bull. Soc. Myc. France **25**: 130. 1909. (FIGS. 54–62)

Trichoglossum hirsutum f. *wrightii* Durand, Ann. Myc. **6**: 438. 1908.

Trichoglossum wrightii Durand, Mycologia **13**: 187. 1921.

Type: On soil, Mt. Koghis, New Caledonia, LeRat no. *122*, in the Farlow Herbarium, Harvard University.

Ascocarps brownish black to black, clavate to broadly spathulate, 1–6 cm long; ascogenous portion flattened, 3–15 mm wide, hard and horny when dried, hirsute from the setae; stipes slender, 1–3 mm thick, hirsute from the setae; asci clavate, 200–255 × 16–24 μ, usually 8-spored, sometimes less; ascospores mostly fusoid-clavate to fusoid, sometimes very variable and abnormal in shape (50–)100–140(–175) × 5–8 μ, 3–9-septate, rarely more, mostly 6–9-septate; paraphyses cylindric, only slightly enlarged above, hyaline or pale brown below, darker above, straight or slightly curved above, adhering and separating with difficulty, slightly projecting above asci, occurring also on stipe; setae abundant, dark brown, acuminate, projecting $\frac{1}{4}$ to $\frac{1}{2}$ of their length beyond the hymenium.

On soil.

Specimens studied: Cuba, C. Wright *382* (FH), *387* type of *T. wrightii* (FH). Bermuda, Britton & Seaver *1404* (NY); Seaver & Waterston (NY); Whetzel, Seaver & Ogilvie (NY, UC).

T. wrightii is also reported from Panama by Nannfeldt.

This species has previously been reported from North America as *T. wrightii*. Durand first described it in his monograph as a form of *T. hirsutum* and later raised it to specific rank. Nannfeldt has suggested that *T. wrightii* and *T. rasum* are the same. A study of the types of *T. wrightii* and *T. rasum* from the Farlow Herbarium supports this view. Both have broadly spathulate ascocarps (FIGS. 58, 59) which are hard and horny, requiring treatment with KOH to separate the asci and paraphyses. In both, the ascospores are very variable in size, shape and septation. Asci with short spores (FIG. 57, 61) occur among the more abundant asci with long spores. While most of the asci have 8 spores some have less, 2–6. Also asci containing abnormal spores having fantastic shapes are found associated with normal asci in both collections (FIGS. 54, 55, 62). The type of *T. rasum* has somewhat more

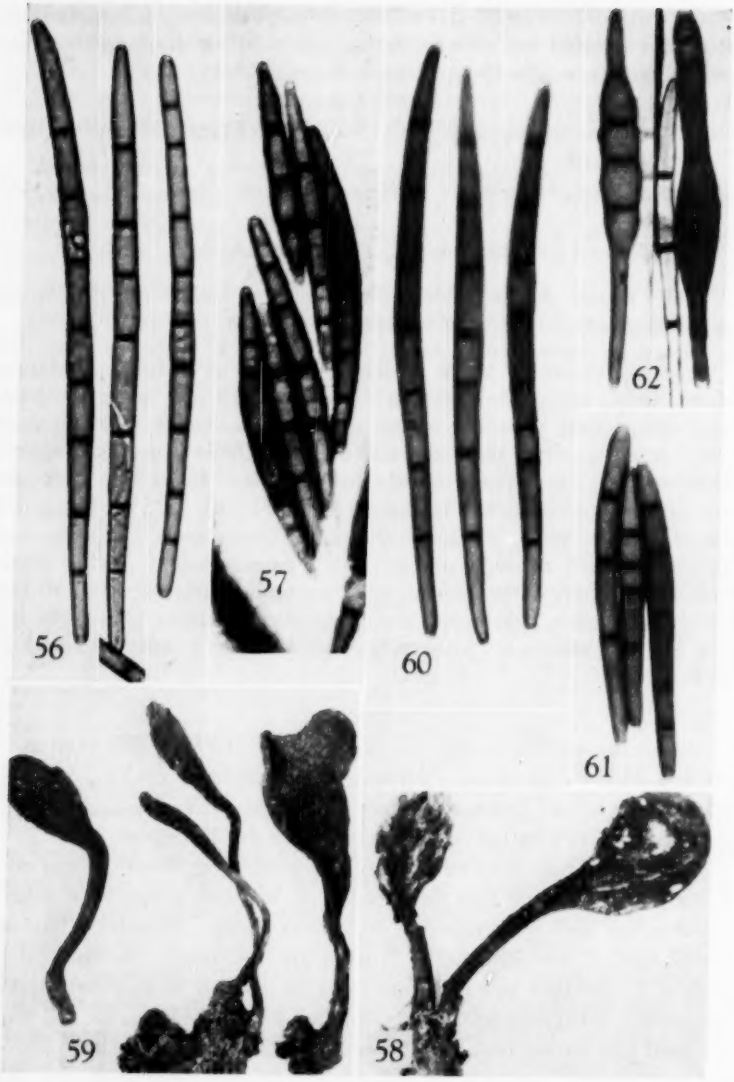


FIG. 56. *Trichoglossum rasum* (*T. wrightii*, type), three long ascospores 8-, 7- and 9-septate; 57, six short ascospores 3–5-septate; 58, two ascocarps × 2; 59–62. *Trichoglossum rasum*, type; 59, four ascocarps × 2; 60, three long ascospores 7- and 8-septate; 61, three short ascospores 3-, 4- and 5-septate; 62, abnormal ascospores. × 600 unless otherwise indicated.

fusoid ascospores than those of *T. wrightii*. On account of the great variability within collections of both *T. wrightii* and *T. rasum* this does not seem significant.

The great variation which occurs even in a single ascocarp, especially the deviations in size, number and shapes of the ascospores, suggests that this species is genetically unstable. Asci with abnormal ascospores occur occasionally in other species of the Geoglossaceae but in several collections of *T. rasum* they occur fairly frequently. The ascospores are mostly 6–9-septate, in this respect resembling *T. velutipes*. It seems doubtful, however, that *T. rasum* and *T. velutipes* are correlated 4- and 8-spored species.

LITERATURE CITED

Boudier, E. 1888. Nouvelles espèces de Discomycètes inoperculés de France. Bull. Soc. Myc. France **4**: 76–88.

Bourdot, H. and A. Galzin. 1927. Hyménomycètes de France. 761 pp.

Coker, William C. 1923. The Clavarias of the United States and Canada. 209 pp.

Cooke, M. C. 1879. New Zealand fungi. Grevillea **8**: 54–68.

Corner, E. J. H. 1950. A monograph of Clavaria and other genera. 740 pp.

Durand, Elias J. 1908. The Geoglossaceae of North America. Ann. Myc. **6**: 387–477.

——. 1921. New and noteworthy Geoglossaceae. Mycologia **13**: 184–187.

Hazslinszky, F. 1881. Rendhagyó köggombák. M. T. Akad. Ert. Term. Kör. **11** (19) : 1–24.

Imai, Sanshi. 1941. Geoglossaceae Japoniae. Jour. Faculty Agr. Hokkaid. Imp. Univ. **45**: 155–264.

——. 1942. Contributiones ad studia monographica Geoglossacearum. Bot. Mag. **56**: 523–526.

Lind, J. 1913. Danish fungi as represented in the herbarium of E. Rostrup. 650 pp.

Luyk, A. van. 1919. Mykologische Remerkungen 1. Geoglossaceen des Reichsherbars zu Leiden. Med. Rijks Herb. Leiden no. **39**: 1–10.

Mains, E. B. 1935. Michigan fungi I. Pap. Mich. Acad. Sci. Arts Letters **20**: 81–93.

——. 1940. New and unusual species of the Geoglossaceae. Am. Jour. Bot. **27**: 322–326.

Nannfeldt, J. A. 1942. The Geoglossaceae of Sweden. Ark. f. Bot. K. Sven. Vetens. **30A** (4) : 1–67.

Patouillard, N. 1909. Champignons de la Nouvelle Calédonie. Bull. Soc. Myc. France **25**: 129–134.

Seaver, Fred J. 1951. The North American cup-fungi (Inoperculates). 428 pp.

Sinden, J. W. and H. M. Fitzpatrick. 1930. A new Trichoglossum. Mycologia **22**: 55–61.

Tai, F. L. 1944. Studies in the Geoglossaceae of Yunnan. Lloydia **7**: 146–162.

Teng, S. C. 1932. Fungi of Chekiang I. Contrib. Biol. Lab. Sci. Soc. China Bot. Ser. **8**: 49–71.

——. 1934. Notes on Discomycetes from China. Sinensia **5**: 431–465.

Winter, G. 1887. Fungi novi Brasiliensis **15**: 86–92.

TROPICAL SOIL FUNGI [1]

W. M. Farrow [2]

This is a preliminary report of some fungi isolated from soils collected in Panama and Costa Rica. Earlier investigations in these areas have been more directly concerned with surface fungi rather than with soil mycobiota. In conjunction with this survey considerable emphasis was also given to selection of nutrient media for isolating the soil fungi. Duplicate soil samples collected from several locations were used for screening nutrient media.

MATERIALS AND METHODS

The soil samples were collected by G. W. Martin and A. L. Welden of the State University of Iowa in July and August, 1952, from six major areas in the Panama Canal Zone and Costa Rica.[3] The soil was taken by scraping the litter surface away with a machete and then thrusting a sterile core tube into the soil as far as practicable. The tubes were plugged and inserted into sterile screw cap test tubes and kept at room temperature until plated. This method of collecting the soil was essentially the same as the one used by Taber (**21**). Often only one or two collections were taken in some areas. These were studied first. In localities where the collections were more numerous and where more or less duplication might be expected, the soil samples were held for later study. Thus, from three to seven months elapsed before it was possible to examine all of the samples. It is reasonable to assume that this prolonged interval proved detrimental to some of the soil organisms, especially those existing in the mycelial condition. Factors such as sensitivity to temperature changes, desiccation and to reduced aeration undoubtedly resulted in loss of some fungi originally present. Table I gives the data on all of the soil samples collected.

Seventeen media were screened in order to select the better ones for this study. The selection of the different substrates for screening was

[1] Based on a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the State University of Iowa, June, 1953.

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based in part on media used in our mycological laboratory, standard media proposed by other investigators and several not suggested in earlier studies on soil fungi. The basic problem was that of finding one or more nutrient media which would support the growth of a wide range of fungi and at the same time restrict the development of the fast-spreading type of fungus.

Martin (**14**) suggested the use of Ha rose bengal-streptomycin agar for isolating soil fungi. This substrate was used primarily as a control in evaluating other media. Triplicate plates were employed with each medium. The addition of streptomycin sulfate to some of the media was necessary to inhibit bacterial growth. The following concentration was used: 1 gram dissolved in 100 cc of distilled water and 8 ml of the solution added to one liter of nutrient medium.

TABLE I

DATA ON SOIL COLLECTIONS

This table gives the general area of each soil sample, the soil type and its pH.

Area	Soil type	pH
1. Barro Colorado Is., Banana soil near Kodak laboratory	mixed clay and humus	6.4
2. Barro Colorado Is., Banana grove	reddish clay	6.2
3. Barro Colorado Is., Termite graveyard	rich humus with only a trace of red clay	6.2
4. Barro Colorado Is., Tower	mixed red clay and humus	6.0
5. Barro Colorado Is., Near laboratory	mixed red clay and humus	6.1
6. Barro Colorado Is., Lutz Trail	rich humus	6.3
7. Barro Colorado Is., Standley Trail	mixed red clay and humus	6.3
8. Barro Colorado Is., Lutz Trail	mixed gray clay and humus	6.4
9. Barro Colorado Is., Locality not recorded	humus and white clay	6.5
10. Barro Colorado Is., Locality not recorded	mixed red clay and humus	6.2
11. Barro Colorado Is., Zetek house	red clay	6.3
12. Barro Colorado Is., Locality not recorded	humus	6.4
13. Barro Colorado Is., Locality not recorded	red clay banana soil	6.3
14. Barro Colorado Is., Bank near Kodak laboratory	red clay	6.3
15. Barro Colorado Is., Donato Trail	rich humus	6.3

TABLE I—*Continued*

Area	Soil type	pH
16. Barro Colorado Is., Banana grove near laboratory	mixed red clay and humus	6.3
17. Barro Colorado Is., Tower	red clay	6.3
18. Barro Colorado Is., Vicinity of laboratory	red clay	6.3
19. Barro Colorado Is., Banana grove	mixed red clay and humus	6.2
20. Barro Colorado Is., Termite graveyard	red clay and humus	6.3
21. Barro Colorado Is., Standley Trail	brook water	6.7
22. Fort Sherman, Canal Zone	red clay	6.2
23. Fort Sherman, Canal Zone	red clay	6.1
24. Rio Sardinilla, Canal Zone	mixed clay and humus	5.9
25. Casita Alta, Chiriqui	rich humus	6.3
26. Casita Alta, Floor beneath house	dry powdered humus	6.4
27. Costa Rica, Crater of Irazu volcano	mixture of powdered rock and humus	6.3
28. Puerto Armuelles, Beneath house	dry powdered humus	6.2
29. Puerto Armuelles, Under mango	rich humus	6.4
30. Puerto Armuelles, Banana plantation	rich humus	7.2
31. Costa Rica, Esquinas Forest	red clay and humus	6.4

The nutrient substrates screened were as follows:

1. Non-carbon nutrient agar (NC)

NH_4NO_3	3	gm
KH_2PO_4	1	gm
$MgSO_4 \cdot 7H_2O$	0.5	gm
KCl	0.5	gm
Agar	15	gm
H_2O (distilled)	1	liter

2. NC with corn stalks

Corn stalks were first placed in petri dishes and sterilized. The nutrient agar was poured over the stalks leaving them about one-half exposed.

3. NC with amino acids

L-Arginine HCl	40	mg
L-Cysteine HCl	40	mg
L-Histidine HCl	40	mg
Non-carbon nutrient agar	1	liter

4. NC with amino acids and corn stalks

L-Arginine HCl	40 mg
L-Cysteine HCl	40 mg
L-Histidine HCl	40 mg
Non-carbon nutrient agar	1 liter

5. NC with thiamin hydrochloride, biotin and corn

Thiamin hydrochloride		40 mg
Biotin solution	0.5 ml	(12.5γ)
Non-carbon nutrient agar		1 liter

6. IIa rose bengal-streptomycin agar

Glucose	10.0 gm
Peptone	2.0 gm
KH_2PO_4	0.5 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
Agar	15.0 gm
Rose bengal	50.0 mg
Streptomycin solution	8.0 ml
H_2O (distilled)	1 liter

7. Hemp agar

Hemp seeds	50 gm
Agar	15 gm
H_2O (distilled)	1 liter

The hemp seeds were added to the water and boiled for 30 minutes. The solution was poured off and several seeds were placed in each petri dish. The hemp seed agar was poured over the seeds leaving only the tips exposed.

8. Dahlia-dextrose agar tablets

Dahlia-dextrose agar tablets	17
Agar	8 gm
H_2O (distilled)	1 liter

9. Dahlia-dextrose agar tablets with *Desmodium* sticks and streptomycin

10. Plain agar with corn stalks and streptomycin

Agar	15 gm
H_2O (distilled)	1 liter

11. Plain agar and cotton thread

Agar	15 gm
H_2O (distilled)	1 liter

Cotton threads were placed in petri dishes and sterilized. The melted agar was then added.

12. Oatmeal agar

Oatmeal	a few flakes per plate
Plain agar solution	20 cc per plate

The plain agar solution was poured over the sterilized oatmeal flakes.

13. Potato-dextrose crystal-violet agar

Potato-dextrose crystal-violet agar tablets	17
Agar	8 gm
H_2O (distilled)	1 liter

14. Potato-dextrose agar tablets and streptomycin

Potato-dextrose agar tablets	32
Agar	8 gm
H_2O (distilled)	1 liter

15. Czapek's agar

$NaNO_3$	3.0 gm
KH_2PO_4	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
KCl	0.5 gm
$FeSO_4 \cdot 7H_2O$	0.01 gm
Sucrose	30.0 gm
Agar	15.0 gm
H_2O (distilled)	1 liter

16. Malt agar

Malt	20 gm
Peptone	5 gm
Agar	15 gm
H_2O (distilled)	1 liter

17. Grass-soil agar

Blue grass (dried)	5 gm
Soil (greenhouse)	10 gm
H_2O (distilled)	1 liter
Agar	15 gm

The grass and soil were boiled separately in 500 cc of water and strained. Both decoctions were mixed and agar added.

Soil dilutions and direct inoculation of the soil were the methods used with several samples. If similar fungi were isolated with both techniques, the more practicable one was used. In preparation for plating, each soil sample was placed in a sterile petri dish and crushed to a fine mixture.

To avoid loss of some fungi which developed late and which might be covered by fast-growing types, all plates in a series were checked frequently under a binocular microscope for a period of two weeks. Few fungi developed after this time, but all plates were held for approxi-

mately one month before discarding. All species of *Penicillium, Aspergillus, Gliocladium, Spicaria* and *Paecilomyces* were subcultured on Czapek's agar to simplify the identification of these fungi.

RESULTS

Several nutrient media were satisfactory for isolating soil fungi. Dahlia-dextrose agar, non-carbon solution agar, or plain agar alone did not contain sufficient nutrients to support the growth of a variable number of fungi. If plant tissues, growth substances, or amino acids were included in these substrates, there was a substantial increase in the kinds of organisms isolated. The inclusion of such constituents in IIa rose bengal-streptomycin agar and hemp agar did not, however, yield a significant number of new fungi. It would be difficult to name the best substrate since they all supported similar kinds of fungi. Some were preferable because they restricted the growth of fast-spreaders, whereas others made possible the isolation of a number of unusual slow-growing types.

Since considerable emphasis was placed on nutrient requirements, some comparison should be made between the different media screened. The advantage of using IIa rose-bengal streptomycin agar were: (1) species of *Aspergillus* and *Penicillium* developed better on this medium than on any of the others tested; (2) the inclusion of rose bengal made it possible to detect growth early; (3) the use of the dye limited colony size, which in turn allowed an increase in the number of fungi on a plate; and (4) bacterial growth was suppressed with the use of rose bengal and streptomycin.

Dahlia-dextrose agar, a commercial product,[4] was more satisfactory for growth if stems of *Desmodium* and streptomycin were added. Such genera as *Dialonectria, Melanospora, Pestalotia* and *Spicaria* grew well on this medium.

The non-carbon solution agar in combination with amino acids, thiamin and biotin, cotton threads, or plant tissues was more favorable for isolating Ascomycetes than were the other substrates tested. The use of amino acids, or growth substances in the non-carbon medium yielded a surprisingly high number of fungi which are perhaps abundant but infrequently isolated from the soil. Some of these were *Chaetomium caprinum, Heterocephalum aurantiacum, Melanospora brevirostris, Melanospora lagenaria, Pseudogymnoascus vinaceus,* and *Tetracoccosporium paxianum.* Frequently, fast-spreading organisms were isolated on substrates containing minute quantities of carbon. Usually, how-

[4] Ben Venue Laboratories, Inc.

ever, the numbers occurring were few when compared with growth on enriched substrates.

The inclusion of dried corn stalks or cotton threads in the non-carbon medium increased the growth rate of several species in the genera *Chaetomium, Chaetoceratostoma, Schizothecium, Melanospora, Sporormia, Thielavia* and *Trichosphaeria.* Fructifications were restricted primarily to the cotton threads or corn stalks rather than on the agar surface, which might suggest that these fungi are capable of utilizing cellulose or other higher carbohydrates present in such material.

The use of a plain agar medium with plant tissues limited the development of most fast-spreading organisms. *Dialonectria, Gliocladium, Phialophora* and *Masoniella* were isolated often on this substrate. A comparison of plant tissues used in a plain agar medium showed that more fungi were isolated with corn stalks than there were using dried *Phleum* or *Desmodium* tissues. However, the last two were better for restricting the growth of fast-spreaders.

Streptomycin was necessary to control the growth of bacteria with a plain agar substrate. The concentration of the antibiotic did not suppress growth entirely but this probably was an advantage to certain Acrasieae and myxomycetes such as *Dictyosteleum, Diderma* and *Didymium* which seem to require bacteria for their development.

Hemp agar was not satisfactory for growth of *Penicillium* and *Aspergillus,* but was excellent for *Graphium, Pseudogymnoascus, Pycnostysanus, Sporormia, Stysanus* and *Thielavia.* Fructifications were much more common on the hemp seed than on the agar surface. Development of fruiting bodies was generally observed only after an extended period of incubation and was apparently correlated with the drying of the seed surface.

The remaining media were rejected because they supported the growth of a very limited number of species, which had been isolated consistently from the selected substrates. Grass-soil agar and oat agar proved more suitable than rich media for obtaining fungi in pure culture. These substrates restricted the growth rates of most fungi, making it possible to isolate an organism with a limited number of transfers. Sugar media were superior for pure culture isolations if the fungus was free from other contaminants.

A total of 135 fungi representing 73 genera was isolated. This number was divided as follows:

Myxomycetes and Acrasiae	3
Phycomycetes	12
Ascomycetes	20
Fungi Imperfecti	100

TABLE II

TOTAL SOIL FUNGI ISOLATED FROM 31 SAMPLES

This table indicates the area from which each fungus was isolated. If the organism was isolated from one or more soil samples, the number is indicated in parentheses.

Fungus	Area					
	Barro Colorado Is.	Casita Alta	Rio Sardinilla	Puerto Armuelles	Costa Rica	Fort Sherman
Absidia glauca Hagem	(6)	(1)	(1)			(1)
Absidia scabra Cocconi	(6)					(1)
Acremonium vitis Cattaneo	(6)	(1)	(1)		(1)	
Acremonium sp.	(3)	(1)		(1)		
Acrostalagmus albus Preuss	(5)	(2)	(1)		(1)	(1)
Acrostalagmus cinnabarinus Corda		(1)				
Alternaria humicola Oudemans	(2)	(1)				
Aspergillus candidus Link	(18)	(1)	(1)	(1)	(1)	
Aspergillus chevalieri (Mang.) Thom & Church	(2)					
Aspergillus flavipes (Bain. and Sart.) Thom & Church	(3)			(1)		
Aspergillus flavus Link	(8)			(1)		
Aspergillus phoenicis (Corda) Thom & Church	(6)			(1)	(2)	
Aspergillus fonsecaeus Thom & Raper	(2)					
Aspergillus fumigatus Fres.					(1)	
Aspergillus giganteus Wehmer	(1)					
Aspergillus niger van Tieghem	(9)		(1)	(2)	(2)	
Aspergillus ochraceus Wilhelm				(1)		
Aspergillus sulphureus (Fres.) Thom & Church	(2)					
Aspergillus sydowi (Bain. and Sart.) Thom & Church	(9)	(1)				(1)
Aspergillus terreus Thom	(1)			(1)		
Aspergillus unguis (Emile-Weil & Gaudin) Thom & Raper	(2)					
Aspergillus ustus (Bainier) Thom & Church	(2)			(1)		(1)
Aspergillus versicolor (Vuill.) Tiraboschi	(8)	(1)		(1)		(1)
Aspergillus violaceo-fuscus Gasperini	(6)			(1)	(1)	
Blakeslea trispora Thaxter	(1)			(1)		
Botryodiplodia theobromae Pat.	(4)					(1)
Botrytis sp.		(1)				(1)
Cephalosporium curtipes Sacc.	(2)					
Cephalosporium sp.	(1)	(1)			(1)	(1)
Chaetoceratostoma sp.	(10)		(1)	(1)		
Chaetomium bostrychodes Zopf		(1)		(1)		
Chaetomium caprinum Bainier	(1)	(1)		(1)		
Chaetomium cristatum Ames	(1)			(1)		
Chaetomium funicolum Cooke	(1)					
Chaetomium globosum Kunze	(3)			(1)		
Chaetomium murorum Corda		(1)				
Chaetomium sp.		(1)		(1)		
Cladosporium herbarum (Pers.) Link	(4)	(1)		(1)		(1)
Coccosporium sp.	(6)	(1)	(1)	(1)		
Coemansia reversa van Tieghem	(1)					

TABLE II—*Continued*

Fungus	Area					
	Barro Colorado Is.	Casita Alta	Rio Sardinilla	Puerto Armuelles	Costa Rica	Fort Sherman
Cunninghamella echinulata Thaxter	(13)	(1)	(1)	(1)		
Curvularia geniculata (Tracy & Earle) Boed.	(2)	(1)				
Curvularia lunata (Walker) Boed.	(6)			(1)		
Dialonectria brassicae (Ellis & Sacc.) Cooke	(2)			(1)		
Dialonectria galligena (Bres.) Petch	(2)		(1)			(1)
Dictyostelium mucoroides Bref.	(4)		(1)	(1)		
Didymium iridis (Ditmar) Fries				(1)		
Diderma sp.	(1)					
Fusarium neoceras Wollenw. & Reink.	(6)			(1)		
Fusarium solani (Martius) Appel & Wollenw.	(14)	(1)	(1)	(3)	(2)	(2)
Fusarium sp.	(2)					
Gliocladium catenulatum Gilman & Abbott	(6)			(1)		
Gliocladium penicilloides Corda	(9)	(1)	(1)		(1)	(2)
Gliocladium roseum (Link) Thom	(2)				(1)	
Gliomastix convoluta (Harz) Mason	(6)			(2)		
Gonytrichum macrocladum (Sacc.) Hughes	(4)					
Graphium bulbicola Hennings	(4)					
Graphium sp.	(4)			(1)		(1)
Helminthosporium sp.			(1)			
Heterocephalum aurantiacum Thaxter	(3)					
Hormodendrum cladosporiodes (Fresenius) Sacc.	(4)			(1)		
Humarina sp.		(1)				(1)
Humicola fuscoatra Traeen	(11)	(2)		(3)		
Melanospora brevirostris (Fuckel) von Höhn.		(1)			(1)	
Melanospora lagenaria (Pers.) Fuckel	(1)					
Memnoniella echinata (Riv.) Galloway				(1)		
Masoniella grisea (Smith) Smith	(4)			(1)		
Monosporium acuminatum var. *terrestre* Sacc.	(2)					
Monotospora brevis (Gilman & Abbott) Mason	(10)		(1)		(1)	(1)
Mucor circinelloides van Tieghem		(1)				
Mucor plumbeus Bonorden	(1)			(1)	(1)	
Mycogone nigra (Morgan) Jensen	(2)					
Nigrospora sphaerica (Sacc.) Mason	(2)				(1)	
Paecilomyces varioti Bainier	(1)					
Patouillardiella sp.	(3)					
Penicillium asperum (Shear) Raper & Thom	(5)	(1)		(1)	(2)	
Penicillium baarnense van Beyma						(1)
Penicillium citrinum Thom	(6)	(1)				(1)
Penicillium decumbens Thom		(1)				

TABLE II—*Continued*

Fungus	Area					
	Barro Colorado Is.	Casita Alta	Rio Sardinilla	Puerto Armuelles	Costa Rica	Fort Sherman
Penicillium fellutanum Biourge	(1)					
Penicillium funiculosum Thom	(4)	(1)	(1)	(2)		(2)
Penicillium humuli van Beyma	(2)					
Penicillium janthinellum Biourge				(1)		
Penicillium javanicum Szilvinyi		(1)				
Penicillium kapuscinskii Zaleski		(1)				
Penicillium lanosum Westling	(2)	(1)		(1)		
Penicillium paxilli Bainier	(9)	(1)		(2)	(1)	
Penicillium raistrickii Smith	(1)					
Penicillium rubrum Stoll	(1)					
Penicillium simplicissimum (Oudemans) Thom	(4)	(1)		(1)		(2)
Penicillium steckii Zaleski	(4)				(1)	(1)
Penicillium stoloniferum Thom		(1)				
Penicillium terlikowskii Zaleski	(2)			(1)		
Penicillium thomii Maire		(1)				
Penicillium vinaceum Gilman & Abbott	(16)	(2)	(1)	(3)	(2)	
Penicillium wortmanni Klocker	(8)		(1)	(1)		
Penicillium sp.		(1)				
Pestalotia sp.	(5)				(2)	(2)
Phialophora sp.	(2)				(1)	
Phymatotrichum sp.	(2)					
Pseudogymnoascus vinaceus Raillo	(3)	(1)				
Pycnostysanus resinae Mason		(1)				
Pythium sp.	(2)					
Rhizopus arrhizus Fischer				(1)		
Rhizopus nigricans Ehrenberg	(1)					
Saksenaea vasiformis Saksena	(1)					
Schizothecium fimicola (Rob.) Griff. & Seaver	(2)	(1)			(1)	(1)
Schizothecium sp.	(2)	(1)				
Spicaria violacea Abbott	(6)				(1)	
Spicaria sp.	(1)	(1)				
Sporormia minima Auerswald	(4)	(1)		(1)		(1)
Stachybotrys cylindrospora Jensen	(1)				(1)	
Syachybotrys lobulata Berk.						(1)
Stemphylium botryosum Wallr.					(1)	
Stysanus medius Sacc.	(4)					
Syncephalastrum racemosum (Cohn) Schroet.	(1)					
Tetracoccosporium paxianum Szabo	(1)					
Thielavia terricola (Gilman & Abbott) Emmons	(2)			(1)		
Thielaviopsis basicola (Berk. & Br.) Ferraris	(3)	(1)		(1)		
Trichocladium asperum Harz		(1)				
Trichoderma koeningi Oudemans	(15)	(2)	(1)	(2)	(2)	(2)
Trichoderma lignorum (Tode) Harz	(10)	(1)	(1)		(2)	(2)
Trichosphaeria sp.	(2)					
Trichosporium sp.	(2)	(1)		(1)		(1)
Verticillium sp.	(2)					
Verticillium terrestre (Link) Lindau			(1)			

TABLE II—*Continued*

Fungus	Area					
	Barro Colorado Is.	Casita Alta	Rio Sardinilla	Puerto Armuelles	Costa Rica	Fort Sherman
Unknown #1 (Sphaeriaceae)	(1)					
Unknown #2 (Dematiaceae)	(1)					
Unknown #3 (Dematiaceae)	(1)					
Unknown #4 (Dematiaceae)	(2)					
Unknown #5 (Moniliaceae)	(1)			(1)		
Unknown #6 (Moniliaceae)	(5)					
Unknown #7 (Moniliaceae)	(2)	(1)				
Unknown #8 (Tuberculariaceae)	(8)					
Unknown #9 (Dematiaceae)	(1)					

No Basidiomycetes were isolated. TABLE II lists the fungi and the area in which each was isolated. Included is a list of unknowns which have been identified to tentative families. Some of these fungi are believed to be undescribed.

DISCUSSION

Most fungi require some source of carbon, nitrogen, minerals, trace elements and growth substances. These constituents were added in some form to the selected media, but there was no criterion, other than the number of fungi isolated, to indicate whether necessary nutrients were included. The isolation of 135 species suggests that growth requirements were fulfilled to a large extent. The number would perhaps have been greater if the soil samples could have been plated immediately after collections were made.

In general, hemp agar and plain agar with plant tissues, both natural media, supported the growth of fewer fungi than the synthetic substrates combined with plant tissues and cotton fibers. While many of the same species were isolated on the selected media, several less common fungi were obtained also, making it imperative to retain all of the substrates.

The multi-point inoculation of soil could be substituted for the dilution method if weak media were used. Preliminary studies had shown that more fungi were isolated with the direct inoculation method. Usually fungi infrequently isolated from the soil would appear on plates inoculated directly, but rarely would be obtained on soil dilution plates.

The growth of typical fast-spreaders was restricted noticeably on media lacking simple sugars. In several instances slow-growing fungi were isolated which, on enriched media, would ordinarily have been

over-grown by other organisms. Also, the development of fructifications was usually more rapid on weak media than on enriched substrates.

Since a greater number of soil samples were collected in Barro Colorado, probably most of the common soil-inhabiting fungi were isolated, whereas the smaller sampling from the other areas would be less indicative of the prevalent fungi. *Penicillium, Aspergillus, Fusarium, Cunninghamella, Trichoderma, Absidia, Chaetomium, Acrostalagmus* and *Gliocladium,* in that order, were the dominant genera isolated from the different localities. *Acrostalagmus albus, Aspergillus candidus, Fusarium solani, Gliocladium penicilloides, Penicillium funiculosum, Penicillium vinaceum, Trichoderma koeningi* and *Trichoderma lignorum* were very common in five of the six areas.

The soils from Costa Rica and Fort Sherman, which were low in humus content, contained high numbers of *Penicillium* and *Aspergillus.* Consequently, it was usually difficult to detect and isolate other fungi growing on the same plates.

Certain Mucorales, such as *Absidia* and *Cunninghamella,* were recorded more frequently than *Mucor* or *Rhizopus.* The latter two have been reported more abundantly from cooler climates. The isolation of 17 species of *Aspergillus* was a number higher than that previously recorded from soil studies of temperate regions. The distribution of *Penicillium* would seem to be independent of climatic conditions.

The majority of fungi isolated have been reported frequently by other workers. However, *Aspergillus fonsecaeus, Aspergillus ochraceus, Aspergillus unguis, Coemansia reversa, Heterocephalum aurantiacum, Masoniella grisea* and *Saksenaea vasiformis* were a few isolated which were rarely or never reported in earlier studies.

Saksenaea vasiformis was isolated from soil in India on several occasions by Saksena (**17**). This fungus produced abundant sporangia on the original medium of amino acids, corn stalks and non-carbon nutrient agar, but subsequent transfers to the same medium and a variety of other substrates failed to induce sporangia development. The following method, suggested by Dr. G. W. Martin, proved successful for obtaining fruiting structures. A species of *Graphium* isolated from Barro Colorado Island was grown on a weak medium until coremia developed. The fungus was then destroyed by steaming at 100° C, and after the agar had solidified, mycelium of *Saksenaea vasiformis* was inoculated at several points around the fructifications. In a week or ten days typical sporangia were observed. The control plates, which had been inoculated with both fungi, showed no evidence of sporangial development after a three-week period.

Masoniella grisea is a very slow-growing fungus which develops late on weak media. It grows in a creeping manner, producing phialids directly on the mycelium or forming short branches which in turn produce one or more phialids. Dark colored, oval spores are formed in chains on these phialids. Frequently the spore chains, phialids and supporting branches resemble a penicillate structure.

Heterocephalum aurantiacum was described by Thaxter in 1904 and not reported, to my knowledge, again until 1951 when Raper and Fennell (**15**) described in detail the unusual development of this fungus from Liberian soil. It is easily recognized when growing on agar because of its protruding structure, size and bright yellow color. This fungus was isolated from three of 21 soil samples from Barro Colorado Island.

Coemansia reversa should be mentioned because it has been reported very rarely from the soil. Only one colony was found growing on the original non-carbon nutrient agar in which corn stalks and amino acids had been added. Several transfers were made to a variety of media, but no growth was observed.

ACKNOWLEDGMENTS

The writer wishes to thank Dr. G. W. Martin for collecting the soil and for his suggestions in preparing this manuscript; to Mr. A. L. Welden for assisting in collecting the soil samples; to Dr. K. B. Raper for identifying several species of *Penicillium;* to Dr. R. F. Cain for examining two species of *Schizothecium;* and to Dr. C. W. Hesseltine for confirming or identifying a number of Mucorales.

SUMMARY

Thirty-one samples were collected from varied soils in Panama and Costa Rica. Because of diversified soil sampling from Barro Colorado Island, many of the more common fungi were isolated. This would be less true of other areas due to fewer collections.

Several media were selected on the basis of a screening process which proved favorable for isolation of soil fungi. Ha-rose bengal-streptomycin agar, hemp agar, non-carbon solution agar with amino acids or growth substances (biotin, thiamin), plain agar and dahlia-dextrose agar were the ones most suitable if using direct inoculation of soil. Addition of dried corn stalks, *Desmodium* twigs or *Phleum* tissue stimulated growth.

One hundred and thirty-five species were isolated, of which 100 were

Fungi Imperfecti. More Ascomycetes were recorded than Phycomycetes.

Nine genera are listed as the most common in the soil samples studied. Species of *Aspergillus* and *Chaetomium* seem to have a wide distribution in this tropical region, whereas *Mucor* and *Rhizopus* were rarely recorded.

The direct inoculation method was practicable if weak media were used. Growth of most fungi was restricted, but not inhibited, on such substrates.

LITERATURE CONSULTED

1. **Bisby, G. R., N. James and M. Timonin.** 1933. Fungi isolated from Manitoba soil by the plate method. Canadian Jour. Res. **8**: 253–275.
2. ——, **M. Timonin and N. James.** 1935. Fungi isolated from soil profiles in Manitoba. Canadian Jour. Res. (sec. C. Bot. Sci.) **13**: 47–65.
3. **Cain, R. F.** 1934. Studies of coprophilous Sphaeriales in Ontario. Univ. Toronto Stud. Biol. Ser. **38**: 1–126.
4. **Chaudhuri, H. and G. S. Sachar.** 1934. A study of the fungus flora of the Punjab soils. Ann. Mycol. **32**: 90–100.
5. **Chesters, G. C.** 1950. Concerning fungi inhabiting soil. Trans. Brit. Mycol. Soc. **32**: 197–216.
6. **Clements, F. E. and C. L. Shear.** 1931. The Genera of Fungi. H. W. Wilson Company, N. Y. 496 pp.
7. **Ellis, E.** 1940. Fungi isolated from pinewood soil. Trans. Brit. Mycol. Soc. **24**: 87–97.
8. **Gilman, J. C.** 1947. A Manual of the Soil Fungi. Iowa State College Press, Ames, Iowa. 392 pp.
9. **Hawker, L. E.** 1948. The effect of certain growth substances on mycelial growth and fruiting of *Melanospora destruens* Shear. Trans. Brit. Mycol. Soc. **31**: 135–141.
10. **Hughes, S. J.** 1951. *Stachylidium, Gonytrichum, Mesobotrys, Chaetopsis* and *Chaetopsella*. Trans. Brit. Mycol. Soc. **34**: 551–576.
11. ——. 1952. *Trichocladium* Harz. Trans. Brit. Mycol. Soc. **35**: 152–158.
12. Imperial Mycological Institute. 1925–1946. Mycological Papers, Nos. 1–15.
13. **Jensen, H. L.** 1931. The fungus flora of the soil. Soil Sci. **31**: 123–158.
14. **Martin, J. P.** 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. **69**: 215–232.
15. **Raper, K. B. and D. I. Fennell.** 1952. Two noteworthy fungi from Liberian soil. Am. Jour. Bot. **39**: 79–87.
16. —— **and C. Thom.** 1949. A Manual of the Penicillia. Williams and Wilkins Co., Baltimore, Maryland.
17. **Saksena, S. B.** 1953. A new genus of Mucorales. Mycologia **45**: 426–436.
18. **Smith, H. R. and V. T. Dawson.** 1944. The bacteriostatic action of rose bengal in media used for plate counts of soil fungi. Soil Science **58**: 467–471.
19. **Smith, G.** 1952. *Masoniella* nom. nov. Trans. Brit. Mycol. Soc. **35**: 237.
20. **Subramanian, C. V.** 1952. Fungi isolated and recorded from Indian soils. J. Madras Univ. Sect. B, **22**: 206–222.

21. **Taber, W. A.** 1951. Studies on soil fungi. Thesis. State University of Iowa. 98 pp.
22. **Thom, C. and K. B. Raper.** 1945. A Manual of the Aspergilli. Williams and Wilkins Co., Baltimore, Md.
23. **Waksman, S. A.** 1917. Is there any fungus flora in the soil? Soil Science **3**: 565–589.
24. ——. 1944. Three decades with soil fungi. Soil Science **58**: 89–115.
25. **Warcup, J. H.** 1950. The soil-plate method for isolation of fungi from the soil. Nature **166**: 117.
26. ——. 1951. The ecology of soil fungi. Trans. Brit. Mycol. Soc. **34**: 376–400.
27. ——. 1951. Soil steaming; a selective method for the isolation of Ascomycetes from soil. Trans. Brit. Mycol. Soc. **34**: 515–518.
28. **Zycha, H.** 1935. Mucorineae. Kryptogamenflora der Mark Brandenburg **6a**: 1–264.

A DESCRIPTION OF A NEW SPECIES OF ACHLYA WITH SOME OBSERVATIONS ON ITS PHYSIOLOGY [1]

A. W. Ziegler and R. H. Gilpin

(with 10 figures)

In April, 1950, the senior author isolated from a soil sample collected in a cypress swamp, a species of *Achlya* which closely resembled *A. tuberculata* (**4**). This form has been kept in culture since that time and studied on occasion. These intermittent studies, combined with some physiological observations, have convinced the authors that this fungus should rank as a new species.

Achlya lobata sp. nov.

Mycelium densum, cultura circa 1–1.5 cm. in seminibus *Cannabis sativae*. Sporangia copiosa in culturus juvenibus, longa, cylindrica, 111–210.9 μ longa et 22–29.6 μ lata. Oogonia (plerumque, cum papillis, 48.1 μ), globosa, papillis obtusis aut simplicibus aut duplicibus. Longitudo papillarum ab 6.7– ad 7.4 μ. Ova eccentrica, globosa, 1–4, plerumque 1–2, 25.9–33.3 μ in diam., plerumque 29.6 μ. Antheridia diclina, densa, manentia usque ad maturitatem ovorum.

Mycelial growth dense, reaching a diameter of 1 to 1.5 cm on hemp seeds in charcoal filtered distilled water. Sporangia abundantly formed in young cultures, long, cylindrical, ranging from 111–210.9 μ long and 22 to 29.6 μ in width. Gemmae consisting of a row of short, slightly swollen cells, the ultimate one tapering, plentiful. Oogonia spherical with blunt single or bi-lobed papillae averaging in size, with the papillae, 48.1 μ. Length of oogonial stalk 1–2 times width of oogonium. Length of papillae from 6.7–7.4 μ. Eggs usually spherical, eccentric, 1–4, mostly 1–2, ranging from 25.9 to 33.3 μ in diameter, mostly about 29.6 μ. Antheridia diclinous, abundantly formed and persisting until eggs are mature.

In soil on hemp seed bait. Collected by the senior author in a cypress swamp, Leon County, Florida, 6 miles west of Tallahassee, Florida on state route 20.

This new fungus closely resembles *A. tuberculata* at first sight, but differs from that species in several respects. A careful study of the

[1] Contribution no. 59, Botanical Laboratory, Florida State University.

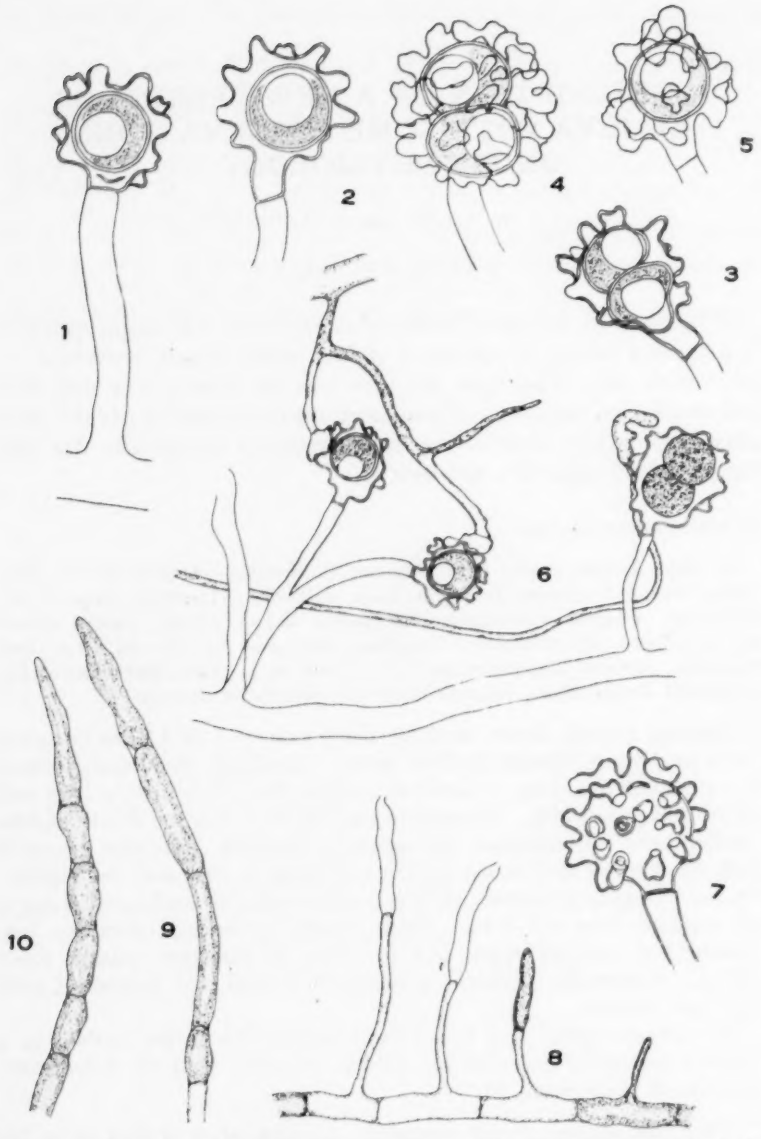

FIG. 1. Oogonium with stalk. Note papillae, × 750. 2. Oogonium with single and bilobed papillae, × 750. 3. Oogonium with double papillae predominating, × 750. 4–5. Oogonia with dense clusters of bilobed papillae, × 750. 6. Habit showing diclinous antheridia, × 450. 7. Empty oogonium showing bilobed papillae, × 750. 8. Germinating gemmae, × 750. 9–10. Gemmae, × 600.

papillae of *A. lobata* shows that the majority of the papillae are larger than those of *A. tuberculata* and usually assume the bilobed condition. One characteristic of *A. tuberculata* is the size variation of its oogonia, which range from 44.4–92.5 μ. This is not observed in *A. lobata*, whose variations extend only from 44.4–59.2 μ, with an average of 48.1 μ in diameter. The number of eggs in *A. lobata* is usually 1–2, while the oogonia of *A. tuberculata* average 3. There is a significant difference in egg size between the two fungi (**4**). Antheridia are rarely formed in *A. tuberculata* but are quite abundant in this new fungus. The eggs do not disintegrate before maturity which is a characteristic of *A. tuberculata* (**4**).

A. lobata may be distinguished from *A. crenulata* by the typical size of its eggs, abundant antheridia, longer non-crenulate papillae, and usual egg number (**3**). It differs from *A. recurva* in oogonial and egg measurements, diclinous antheridia, and the character of the protuberances (**1**). It is separated from *A. abortiva* var. ***normalis*** by its egg size, profuse antheridial development and usual egg number (**2**). *A. lobata* differs from *A. subterranea* in the smaller size of its oogonia, lesser number of eggs per oogonium, diclinous antheridia, and its longer, more prominent oogonial papillae (**2**).

The papillae of this new form are unique. In some instances the bilobed condition is evident on only a few of the protuberances (FIG. 2). On other oogonia all the papillae assume the bilobed appearance (FIG. 4). Some of the lobes exhibit shallow bifurcations, while at other times the indentations are quite pronounced (FIGS. 1, 7). Relatively few papillae may occur on oogonia (FIG. 2) or the oogonium may be so densely clustered with protuberances that the eggs are difficult to see (FIG. 4). All variations may be present in a single culture. In the experiments on physiological behavior noted below, there was no variation from these observations whenever oogonia occurred.

As mentioned before, this fungus was collected in April 1950 and has been kept in culture to the present time. In order to ascertain the constancy of the key characteristics, a series of experiments has been performed. These experiments, with their results, are as follows:

GROWTH ON VARIOUS FOODS

Different food sources were utilized to determine whether the characteristics remained constant while varying the food source. On radish, lima bean, pea, and green bean seeds vegetative growth was sparse and no sexual organs were produced. Hemp seeds produced normal cultures. Barley and corn fruits produced moderately abundant vegetative

growth and sex organ formation was normal. Cultures were grown at room temperature.

GROWTH IN VARIOUS pH CONCENTRATIONS

A phosphate buffer series in double distilled water ranging from 4.6 to 8.0 was utilized. Baited hemp seeds were placed in the solutions and when growth had occurred on other seeds present, the original culture was removed. Room temperature was used.

As can be seen from the table, vegetative growth was abundant in all cultures except in the 4.6, 7.5 and 8.0 pH ranges. The greatest number

TABLE I

pH	Veg. growth	Oogonia and eggs	Gemmae
4.6	Scanty	None	Very abd. gemmae
5.0	Abd.	Few; normal	Very abd. gemmae
5.5	Abd.	Mod. abd.; normal	Very abd. gemmae
6.0	Abd.	Abd.; normal	Very abd. gemmae
6.5	Abd.	Abd.; normal	None
7.0	Abd.	Very abd.; normal	Scanty gemmae
7.5	Mod. abd.	None	None
8.0	Scanty	None	None

of oogonia with normal eggs occurred at the 7.0 pH concentration. Sex organs were not formed in cultures over pH 7.0 and below 5.0. Abundant gemmae were formed in the lower portion of the range, but declined in numbers as the pH approached neutrality. Whenever oogonia were formed, there was no change in size, type of papillae, egg size or number, or antheridial characteristics.

RESPONSE TO TEMPERATURE

Young cultures on hemp seeds, not older than 72 hours, were placed in charcoal filtered distilled water at the temperatures indicated below. These were removed after two weeks and examined.

TABLE II

Temp.	Veg. growth	Oogonia and eggs
5° C	None	None
15° C	Abd.	Very abd.
20° C	Very abd.	Very abd.
23° C	Mod. abd.	Abd.
28° C	Slight	Scanty
33° C	None	None

The best vegetative growth and production of oogonia appeared in the 15–20° C range. The characters which delineate the form were constant throughout the various temperatures whenever oogonia were formed.

MISCELLANEOUS

Cultures grown to maturity in both constant light and in constant darkness showed no variation from the normal. Intermittent light and darkness produced no visible morphological changes.

Cultures grown in an atmosphere containing a large percentage of carbon dioxide did not live. Those in an atmosphere of oxygen, the concentration of which could not be measured, were normal in all respects.

In all the experiments noted above, the key characteristics of this species remained constant. There has been no indication that this form is merely a variety of *A. tuberculata* or *A. crenulata.* On Feb. 11, 1953, this fungus was again isolated from soil collected in a roadside ditch near Clewiston, Florida. The two collections are identical. The authors feel that the establishment of this fungus as a new species, *Achlya lobata,* is justified.

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The authors are indebted to Dr. Francis Walton of the Classics department for the Latin description and to Dr. S. L. Meyer for his reading of the manuscript. The use of equipment granted the senior author under Public Health Grant *G 3710* is also gratefully acknowledged.

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LITERATURE CITED

1. **Coker, W. C.** The Saprolegniaceae. Chapel Hill, N. C., University of North Carolina Press. 1923. 201 pp.
2. —— **and H. H. Braxton.** New water molds from the soil. Jour. Elisha Mitch. Sci. Soc. **42**: 139–149. 1926.
3. **Ziegler, A. W.** A new species of *Achlya.* Mycologia **40**: 336–341. 1948.
4. ——. A new *Achlya* from Florida. Mycologia **42**: 658–662. 1950.

THE PERFECT STAGES OF MARSSONINA RHABDOSPORA AND SEPTOGLOEUM RHOPALOIDEUM [1]

G. E. Thompson

(with 11 figures)

This paper is a report of investigations on the life histories of *Marssonina rhabdospora* (Ellis & Ev.) Magn. and *Septogloeum rhopaloideum* Dearn. & Bisby, with descriptions of their perfect stages. Most of the observations were made when the writer was a graduate student in the Department of Botany, University of Toronto, Toronto, Canada, and the Department of Plant Pathology, Cornell University, Ithaca, New York.

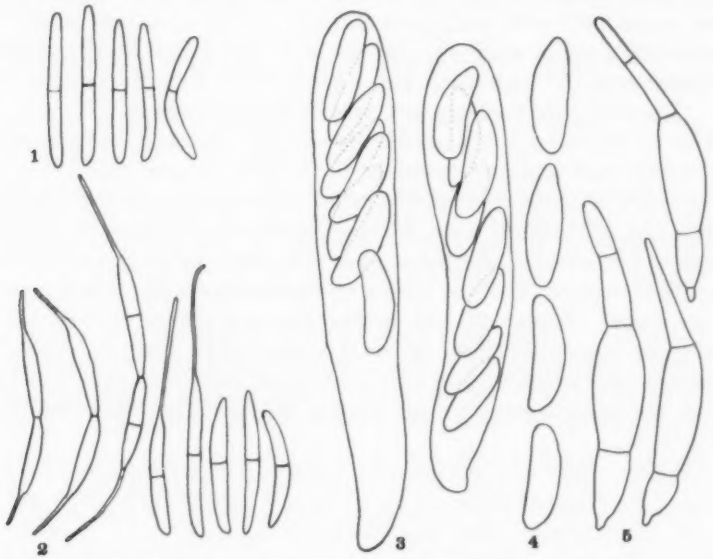
I. Marssonina rhabdospora

Conidial Stage. The conidial stage of this fungus is classified in the *Melanconiales* of the Fungi Imperfecti and was described under two different names in 1893. Ellis and Everhart (**6**) collected it on leaves of *Populus grandidentata* Michx. at Newfield, New Jersey and named it *Marsonia rhabdospora,* while Peck (**9**) described it as *Gloeosporium populinum* from material collected on the same species at Freeville, New York. In 1906, Magnus (**8**) transferred the former to the genus *Marssonina* Fischer. Recently, Vassiljevsky and Karakulin (**10**) have placed it in the genus *Cylindrosporium* under the name *C. populinum* (Pk.) Vassil. Davis (**4**) was apparently the first to record its occurrence on *P. tremuloides* Michx. The writer has made several collections on both hosts in the vicinity of Ithaca, New York, including the area in which Peck made his collections. In addition to the above mentioned States, it has been reported from Pennsylvania and Wisconsin (**11**) and is known to occur on the same hosts at several locations in Ontario, Can-

[1] The writer wishes to thank Dr. Roy L. Cain, Department of Botany, University of Toronto, Toronto, Canada; Dr. I. L. Conners, Department of Agriculture, Ottawa, Canada; Dr. J. Dearness, London, Ontario, Canada; Dr. H. D. House, New York State Museum, Albany, New York, for information and certain specimens of the fungi described in this paper and Dr. A. Kaplan of the Classics Department, University of Georgia, for assistance with the Latin diagnoses.

ada. The fungus is associated with small, circular, brown, zonate spots in the living leaves during the summer and early fall.

The acervuli develop within the lower epidermal cells of the leaf and when mature measure 80–176 μ in diameter (FIG. 6). They are frequently arranged in one or more concentric rings, and, when moist, they are filled with prominent flesh-colored masses of conidia. The conidia, which measure 22.5–35 × 2.5–3 μ, are cylindrical with rounded ends, straight or slightly curved, equally two-celled and hyaline (FIG. 1).



FIGS. 1, 2. *Pleuroceras populi*. 1. Conidia. 2. Stages in the development of ascospores, from immature spores on the left to mature ones on the right, × 800. FIGS. 3–5. *Guignardia populi*. 3. Asci with ascospores. 4. Ascospores. 5. Conidia, × 650.

Other species of *Marssonina* which occur on the leaves of poplars have ovate or pyriform, unequally 2-celled, hyaline conidia.

Spermatial Stage. Davis (**3**) reported a spermatial stage of this fungus on the leaves of *P. grandidentata* collected in September. He described the spermatia as being abstricted from erect, straight hyphae in acervuli, hyaline, filiform, 7–11 × 2 μ. None of the writer's collections show this stage, probably because they were not made late enough in the season when spermatia are characteristically produced by many leaf-spotting fungi.

Perfect Stage. During the fall of 1936, leaves of *P. tremuloides* bearing acervuli with conidia were collected at Ithaca and Freeville, New York, and placed in wire baskets for wintering out-of-doors. When these leaves were examined in April and May, 1937, beaked perithecia with asci and ascospores were found. The thin-walled, membranaceous perithecia were embedded obliquely in the mesophyll of the leaf. In longitudinal sections, they were pyriform in shape and measured 128–160 × 160–220 μ. The beaks arose laterally from the perithecia and protruded through the leaf up to 250 μ in length. The canals of the beaks were lined with periphyses (FIG. 7). When mature perithecia were crushed out in water, the fusiform asci, 144–217 × 16–19 μ, floated out separately and each ascus had a conspicuous refractive ring at the tip. The asci were 8-spored and paraphyses were absent.

The young ascospores were 2-celled, hyaline, fusiform, constricted at the septum and had an appendage at the end of each cell (FIG. 2). Later, each cell was divided by another septum without any constriction. The appendages were flexuous, thread-like and up to 40 μ in length. The mature ascospores were 3-septate, with a constriction at the middle septum, 48–70 × 3.5–4 μ in size and lay more or less parallel in the ascus. When asci with mature ascospores were crushed out in water, the spores usually broke apart at the middle septum. This was especially true when mounts were made from dried material. Furthermore, the appendages were not readily visible under these conditions (FIG. 2).

The above features of the fungus are characteristic of the genus *Pleuroceras* of the *Gnomoniaceae*. This genus and its relation to other genera of the *Gnomoniaceae* have been discussed by v. Arx (1), v. Arx and Olivier (2) and v. Höhnel (7). The only previously described species of this genus which occurs on the leaves of poplars is *Pleuroceras cryptoderis* (Lév.) v. Höhn. (*Cryptoderis lamprotheca* (Desm.) Auers.) which occurs on *P. alba* L. in Europe. The writer has examined one European specimen labelled *C. lamprotheca* (Linhart Fungi Hungarici No. *369*). In making mounts from this material, the ascospores when crushed from the asci were always separated at the middle septum so that it was impossible to obtain measurements of entire spores. The segments measured 19–22.5 × 3–3.5 μ as compared with 25–40 × 3.5–4 μ for similar segments of ascospores of the fungus under consideration. The ascospore measurements as given in the description of *C. lamprotheca* are 42–44 × 3 μ as compared with 48–75 × 3.5–4 μ for the fungus being described in this paper.

In view of the differences in ascospore sizes between the European

fungus and the American and the fact that the conidial stage is known only in North America on *P. grandidentata* and *P. tremuloides* and not on *P. alba,* it is therefore being described under the following name and diagnosis.

Pleuroceras populi sp. nov.

Marsonia rhabdospora Ell. & Ev. Proc. Phil. Acad. Nat. Sc. 168. 1893.

Gloeosporium populinum Pk. Ann. Rept. New York State Bot. **45**: 20. 1893.

Marssonina rhabdospora (Ell. & Ev.) Magn. Hedwigia **45**: 88–91. 1906.

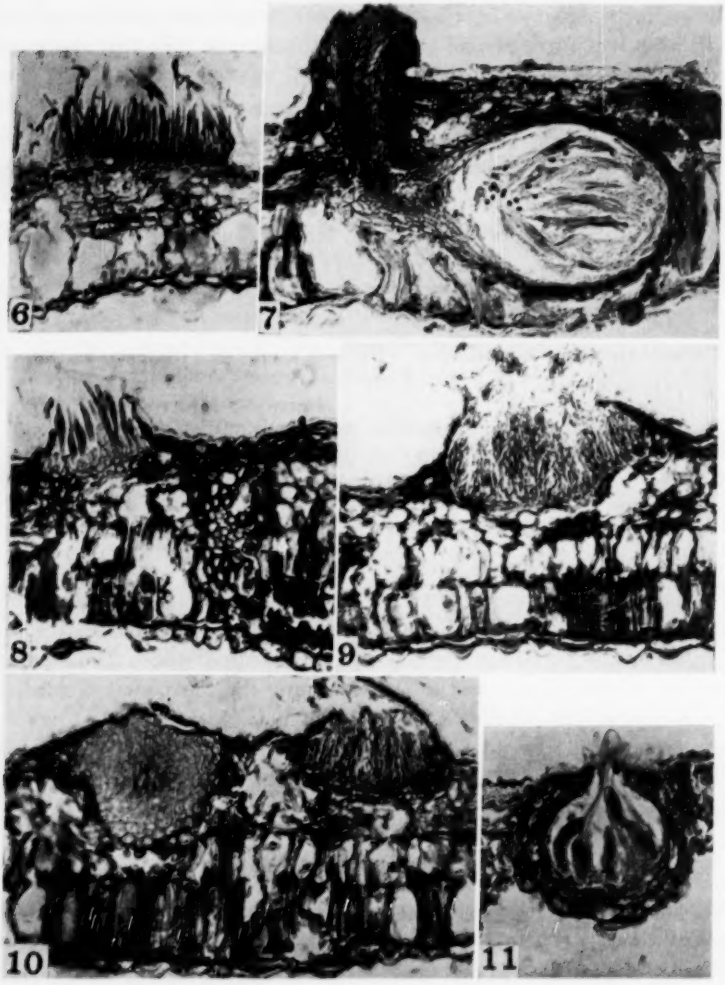
Cylindrosporium populinum (Pk.) Vassil. F. Imperf. Paras. 11. Melanconiales. P. 575. 1950.

Perithecia sparsa, innata, pyriformia, incumbentia, pariete membranaceo, 128–160 × 160–220 μ, aparaphysata, rostro laterali, perforante hypophyllo vel epiphyllo, 150–250 μ longo; ascis fusoideis, attenuatis, 144–217 × 16–19 μ, octosporis; sporidiis fusoideo-elongatis, stipatis, triseptatis, medio constrictis, rectis vel curvatis, hyalinis, 48–70 × 3.5–4 μ, utrimque appendiculatis.

Cultures. In the spring of 1937, small pieces of wintered leaves of *P. tremuloides* bearing mature perithecia were suspended above the surface of potato-dextrose agar in Petri dishes. The ascospores, discharged on the surface of the medium, germinated within 24 hours and transfers were made to test tubes containing the same kind of medium. In two weeks the colonies were 2–3 mm in diameter, light brown and firm in texture. Conidia typical of those which occur in nature on infected leaves were found, thus establishing the relationship of the sexual stage with the asexual stage.

II. SEPTOGLOEUM RHOPALOIDEUM

Conidial Stage. Septogloeum rhopaloideum was described by Dearness and Bisby (**5**) in 1928 on living leaves of *P. tremuloides* in Manitoba, Canada. The writer collected this fungus several times on the above host in the Lake Temagami district of Ontario, Canada, and from numerous localities near Ithaca, New York. It has also been reported from Wyoming(**11**). One collection was of especial interest. It was labelled "*Septogloeum* on *P. balsamifera,* Elka Park, Greene Co., New York, Sept., 1908, H. S. Jackson." Conidia and spermatia were present in the spots on the leaves and were identical with those of *S. rhopaloideum* on *P. tremuloides.* The occurrence of the fungus on this host



FIGS. 6, 7. *Pleurocceras populi.* 6. An acervulus with conidia. 7. A perithecium showing lateral beak lined with periphyses and asci with refractive ring at the tips. FIGS. 8–11. *Guignardia populi.* 8. An acervulus with conidia. 9. An acervulus with spermatia. 10. A perithecial primordium (left) adjacent to an acervulus with spermatia. 11. A mature perithecium with asci, × 250.

apparently has not been reported previously. The fungus is associated with greyish or brownish, circular or irregular spots which frequently coalesce to form extensive dead areas in the leaves.

The acervuli are initiated subepidermally on the underside of the spots and when mature measure 95–144 μ in diameter (FIG. 8). The conidia are hyaline, 2–4-septate and are described as "Indian-club or tadpole shaped." The basal cell of the conidium is short and broad with a nipple at the tip. Occasionally a septum is formed between the nipple and basal cell. The middle cell is relatively large and barrel-shaped, while the terminal cell is attenuated and rounded at the tip and occasionally 1-septate (FIG. 5). The conidia measure 64–80 $\times$ 9.5–12.5 μ.

Spermatial Stage. This stage of the fungus apparently has not been reported previously. The hyaline, 1-celled, rod-shaped spermatia, 5–7 $\times$ 1.5–2 μ, are budded off from the ends and sides of well developed, septate spermatiophores in acervuli. The latter are hypophyllous, 95–144 μ in diameter (FIG. 9). In the early part of September, these were intermingled with acervuli bearing conidia, whereas in October practically all acervuli produced spermatia. From stained preparations of material fixed at different dates during the late summer and fall, the same acervuli which produced conidia during the summer appeared to produce spermatiophores with spermatia in the fall. In some cases, the conidia may actually become slightly modified and function as spermatiophores.

Perfect Stage. The perithecial primordia begin their development during late September and October. At that time, they are present on the lower surface of the leaf spots and are intermingled with the spermatial acervuli. They continue their development during the fall and mature in the spring (FIGS. 10, 11).

Mature perithecia are closely crowded together in localized areas. They are depressed-globose in shape, almost black in color and measure 114–152 $\times$ 95–114 μ. The perithecial walls are composed of 2–3 layers of thick-walled, pseudoparenchymatous cells. The ostioles are simple. The clavate-cylindrical, 8-spored asci cling together in fascicles when crushed from perithecia in water mounts. They measure 70–112 $\times$ 16–19 μ. The ascospores are arranged biseriately in the asci and are elliptical to oblong in shape, straight or slightly curved, hyaline and 1-celled, measuring 22–33 $\times$ 6.5–8 μ. Paraphyses are absent (FIGS. 3, 4).

This stage of the fungus is placed in the genus *Guignardia* of the family *Mycosphaerellaceae* on the bases of the fasciculate asci, 1-celled ascospores and the absence of paraphyses. The only member of this

genus which has been reported as occurring on the leaves of poplars is a European species, *Guignardia niessli* (Kze.) Lindau, which has not been reported in North America. However, the writer has collected it on wintered leaves of *P. tacamahaca* Mill. in the Lake Temagami district of Northern Ontario. This species has much smaller ascospores, 9.5–14 × 3.5–4.5 μ as compared with the American fungus, which has ascospores 22–33 × 6.5–8 μ. Other differences between the two fungi are the hosts and conidial stages, none having been reported associated with *G. niessli*. In view of these facts, it is described as a new species with the following name and diagnosis:

Guignardia populi sp. nov.

Septogloeum rhopaloideum Dearn. & Bisby, Mycologia **20**: 243. 1928.

Perithecia hypophylla, dense gregaria, nigra, depresso-globosa, 114–152 × 95–114 μ, aparaphysata, pariete pseudoparenchmatico, ostiolo plano; ascis fasciculatis, clavatis vel cyclindricis, 70–112 × 16–19 μ, octosporis; sporidiis distichis, elliptico-oblongatis, rectis vel curvatis, continuis, hyalinis; 22–33 × 6.6–8 μ.

Since no cultural work on the fungus has been done, the relationship of the various stages is based on the fact that they are constantly associated and develop in chronological order throughout the year.

Specimens from the type collections of both species of fungi have been deposited in the following herbaria: Mycological Herbarium, Department of Agriculture, Ottawa, Canada; Department of Botany, University of Toronto, Toronto, Canada; Department of Plant Pathology, Cornell University, Ithaca, N. Y., and Mycological Collections of the U. S. D. A. Bureau of Plant Industry, Beltsville, Maryland.

SUMMARY

The perfect stage of *Marssonina rhabdospora* (Ell. & Ev.) Magn. which occurs on the leaves of *P. grandidentata* and *P. tremuloides* is described as a new species under the name *Pleurocera populi*. Cultures obtained from ascospores produced conidia which were typical of those found in nature on the host plants.

The perfect and spermatial stages of *Septogloeum rhopaloideum* Dearn. & Bisby, which occur most commonly on *P. tremuloides*, are described, and *P. tacamahaca* (*P. balsamifera*) is reported as an additional host. The name *Guignardia populi* is given to the perfect stage.

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LITERATURE CITED

1. **Arx, J. A. von.** Über die Gattung *Lacstadia* and *Gnomoniaceen*. Antonie Van Leeuwenhoek Jour. Micro. and Serol. **17**: 259–272. 1951.
2. ——, **and Dorothea L. Olivier.** The taxonomy of *Ophiobolus graminis* Sacc. Trans. Brit. Myc. Soc. **35**: 29–33. 1952.
3. **Davis, J. J.** *Marssonina rhabdospora* (Ell. & Ev.) Magn. Parasitic fungi in Wisconsin. Trans. Wis. Acad. Sci. Arts and Letters **18**: 103. 1915.
4. ——. *Marssonina rhabdospora* (Ell. & Ev.) Magn. Parasitic fungi in Wisconsin. Trans. Wis. Acad. Sci. Arts and Letters **21**: 257. 1924.
5. **Dearness, J.** New and noteworthy fungi V. Mycologia **20**: 243. 1928.
6. **Ellis, J. B. and B. M. Everhart.** *Marsonia rhabdospora* E. & E. New species of North American fungi from various localities. Proc. Phil. Acad. Nat. Sci. 168. 1893.
7. **Höhnel, F. von.** Mycologische fragmente. Ann. Myc. **16**: 101. 1918.
8. **Magnus, P.** Notwendige Umänderung des Namens der Pilzgattung *Marssonia* Fischer. Hedwigia **45**: 88–91. 1906.
9. **Peck, C. H.** *Glocosporium populinum* n. sp. Ann. Rept. New York State Bot. **45**: 20. 1893.
10. **Vassiljevsky, N. I. and B. P. Karakulin.** Fungi Imperfecti Parasitici, Pars. 11. Melanconiales. 575. Inst. Bot. Acad. Sci. 1950.
11. **Weiss, Freeman and Muriel J. O'Brien.** Index of plant diseases in the United States. Plant Disease Survey Special Publication No. 1. Part V. 1039–1040. 1953.

A NEW GENUS OF MONILIACEAE

S. B. Saksena

(with 10 figures)

During the course of his studies on soil fungi of Sagar the writer isolated this interesting fungus from a soil sample from Ghatera forests —a spot about 60 miles east of Sagar in the state of Madhya Pradesh, India.

MATERIAL AND METHODS

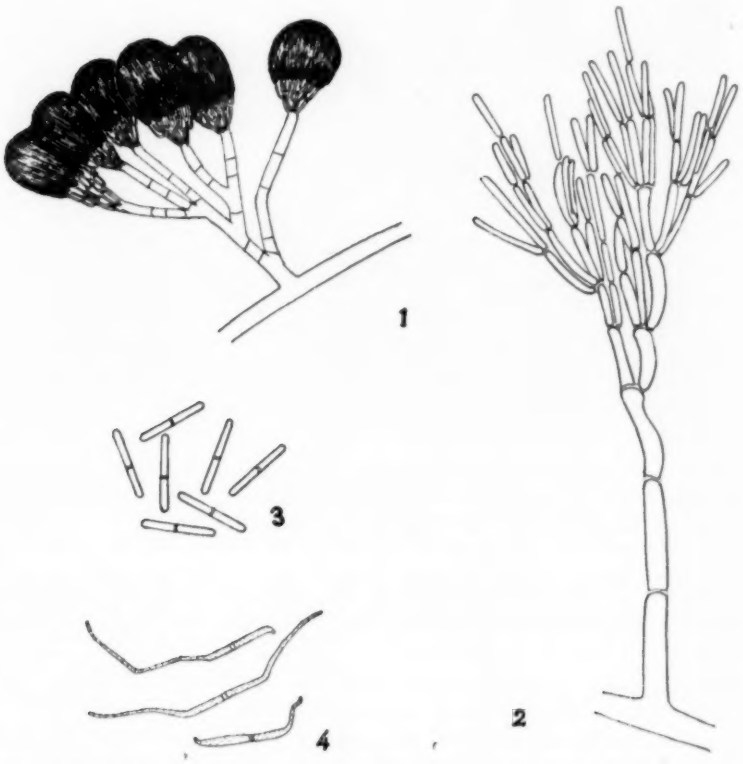
The soil was collected in September, 1952, from a depth of 2–4 inches with a sterile spatula, packed in a sterile container and brought to the laboratory. The soil was plated in three different dilutions in Petri dishes of several media. The fungus first appeared on potato dextrose agar. Further isolation and purification was done on Czapek's agar and detailed observations have been recorded on the same. The fungus was later tried on various other media, *viz.* soil extract agar, malt agar, potato dextrose agar and Waksman's agar for recording the behavior and cultural characteristics.

GENERAL STRUCTURE

The most remarkable features are the pattern of the conidiophores and conidia. The conidia are 2-celled, very thin and cylindrical, measuring 18–24 × 1.5–2 μ. Both the ends are rounded and the septum is exactly in the middle (Figs. 3, 9). The conidiophores are much branched and the ultimate branches develop into definite penicillus heads (Figs. 2, 7, 8). The conidia are produced abundantly in succession at the tips of the final branchlets (phialides) but they do not form chains. They accumulate in slime and form balls. The balls of the neighboring heads coalesce to form bigger ones (Figs. 1, 6).

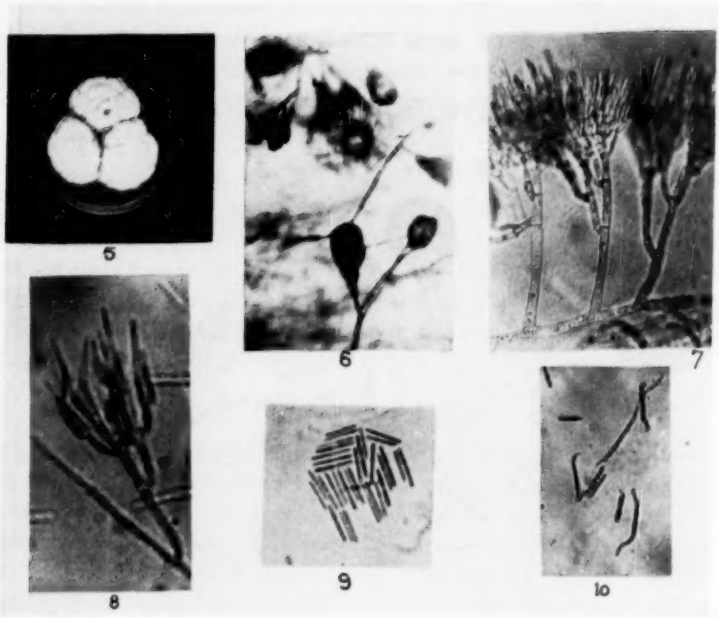
Since the conidiophores are scattered all over the surface of the colony and the color in mycelium, conidiophores and conidia is clear and bright, the fungus clearly belongs to the family Moniliaceae of the Fungi Imperfecti. The development of the conidial apparatus would show an affinity with the genus *Penicillium*. The absence of chains of conidia and

their accumulation in slime balls shows a close resemblance with the genus *Gliocladium* so far as the habit is concerned. But the shape and structure of the conidia are clearly different from the 1-celled roundish, oval, or short-cylindrical conidia which usually occur in the above two genera.



FIGS. 1–4. *Glococladiopsis sagaricnsis*. 1. Habit, × 150. 2. Details of branching and formation of conidia, × 600. 3. Conidia, × 600. 4. Germination of conidia, × 600.

The shape of the conidial apparatus also appears to be a little different from that described for *Gliocladium*. There appears to be a greater degree of branching and the branches are not as appressed as in *Gliocladium*. The fungus is, therefore, described as a new genus. It is being named *Gliocladiopsis* due to its superficial resemblance with *Glio-*



FIGS. 5–10. *Glococladiopsis sagariensis*. 5. Ten days' growth on Czapek medium. 6. Habit, × 100. 7. Details of branching and formation of conidia, × 330. 8. Same, × 490. 9. Conidia, × 315. 10. Germination of conidia, × 315.

cladium and the species, which is the only one known at present, is named *Gliocladiopsis sagariensis* after the locality i.e., the Sagar district.

Gliocladiopsis gen. nov.

Conidiophori ramosi, parietibus levibus praediti, septati, in capita panicillata desinentes. Fructificationes ut plurimum constantes ramis primariis, secondariis atque phialidibus. Conidia tenuia, cylindrica, acicularia, bicellulata, septo distincto ad medium, successive producta, in massas mucosas ad apices capitum penicillatorum aggregata.

Conidiophores branched smooth walled, septate, ending in definite panicillus heads. Fructifications usually in three to four stages consisting of primary branches, secondary branches and phialides. Conidia thin, cylindric (acicular), two celled with a clear septum just in the middle, produced in succession, becoming aggregated in slimy masses at the apices of the panicillus heads.

Gliocladiopsis sagariensis sp. nov.

Coloniae in medio Czapek cultae primo albidae, floccosae et diffusae, incremento cito ad 8 cm diam. diebus 10 obtento. Cultura transparens hypharum sterilium producit hyphas flocculosas aereas, quae efformant structuras funi similes. Conidiophori exsurgunt directe ex mycelio sterili prostrato vel ut surculi laterales ex hyphis aereis flocculosis. Ut conidiophori ad maturitatem accedunt, coloniae assumunt primo colorem pallide viridi-luteum, tum transeunt per cremeo-luteum, in armeniacum. In maturitate coloniae evadunt acute zonatae et monstrant areas conidiales coloratas alternantes cum areis sparse fertilibus, quae sunt fere transparentes. Pars aversa albida primo, evadens pallide lutea ut fructus evolvitur, tandem pallide armeniaca. Coloniae plene evolutae monstrant zonas distinctas etiam in parte aversa. Conidiophori ramosissimi, parietibus levibus praediti, septati, longitudinis varibilis usque ad 350 μ; rami ultimi evolvunt capita penicillata. Fructificationes ut plurimum monstrant ramos primarios 14–22 × 3–4 μ, secondarios 8–12 × 2–3 μ, atque phialides 10–15 × 2 μ. Conidia tenuia, cylindrica (acicularia), bicellulata, septo claro ad medium, 18–24 × 1.5–2 μ, parietibus levibus praedita, apicibus rotundatis. Conidia atque conidiophori tenuiter lutea, colore in massis prominente. Conidia abundanter producta in successione ex phialidibus, evadunt aggregata in massas mucosas ad apices capitum penicillatorum. Massae conidiales ex pluribus capitibus unitae ad massas efformandas ampliores.

Colonies on Czapek's agar at first white, floccose and spreading, growth medium fast attaining the diameter of 8 cm in 10 days. Transparent growth of sterile hyphae gives off the flocculent aerial hyphae which form rope-like structures. Conidiophores arise either directly from the prostrate sterile mycelium or as the side shoots from the flocculent aerial hyphae. As the conidiophores begin to mature the colony assumes at first a light greenish yellow color (Pl. 10—F1) later changing through creamy yellow (Pl. 9—G3) finally to apricot color (Pl. 10—F7). At maturity the colony becomes sharply zonate showing the colored conidial areas alternating with the sparsely fertile areas which are almost transparent. Reverse white at first, becoming faintly yellow (Pl. 10—D1) as fruiting develops, later becoming light apricot (Pl. 11—G5). Fully developed colonies show sharp zonations on the reverse as well. Conidiophores much-branched, smooth-walled, septate structures of variable length up to 350 μ; the ultimate branches developing into definite penicillus heads. The fructifications are usually in three to four stages consisting of primary branches 14–22 × 3–4 μ; secondary branches 8–12 × 2–3 μ; and phialides 10–15 × 2 μ. Conidia thin, cylindric (acicular), 2-celled, with a clear septum just in the middle, 18–24 × 1.5–2 μ, smooth-walled with rounded ends. The conidia and the conidiophores slightly yellowish, the color becomes pronounced in masses. Conidia produced abundantly in succession from the phialides, becoming aggregated in slimy masses at the apices of the penicillus heads. Conidial masses from several heads fusing to form bigger masses.[1] Culture deposited with Indian Type Culture Collection, New Delhi.

[1] The color plates referred to are from Maerz & Paul's Dictionary of Color.

CULTURAL CHARACTERISTICS

The fungus was grown on various culture media to ascertain its behavior and peculiarities. The following observations have been recorded:

Soil agar: Radial growth quite fast, rather faster than on other richer media, but branching sparse so that the thick flocculence did not develop. Zonations well-marked. Very little coloration developed even in the older colonies. The conidiophores and conidia acquired the same size except that the number of branches in the conidiophores and the total quantity of conidia per head were much diminished. The conidiophores were sparsely spread.

Waksman's agar: The colony showed almost the same characteristics as noted for Czapek's agar excepting that some radial furrows could be noted on the reverse. The color succession and measurements remained the same.

Potato dextrose agar: The general characteristics of the colony were the same as recorded for Czapek's medium except that the growth was more luxuriant and showed profuse development of flocculent hyphae. Colors and measurements were the same.

Malt agar: General observations recorded were the same. The color, however, was markedly deeper. Growth was luxuriant with profuse development of flocculent hyphae. Measurements were the same.

SPORE GERMINATION

Spore germination was tried in pea decoction. The spores readily germinated in 4–5 hours. During the process, the spores first imbibed water and swelled and the middle septum became more distinct. Germ tubes were produced from the two ends (FIGS. 4, 10) and grew rapidly producing the septate hyphae. The spores which produced hyphae at their ends themselves became an integral part of the developing hyphae and lost all identity. Sometimes germ tubes were given out from the sides of the spores.

AFFINITIES AND DISCUSSION

Clearly, the fungus with its habit and the typical conidial apparatus resembles *Penicillium* and *Gliocladium*. From *Gliocladium* it differs in having a different type of spore and in certain details of the conidiophores.

The genus also shows superficial resemblance with *Cylindrocladium* (type species *C. scoparium* Morgan, 1892) in general structure of conidiophore and the 2-celled cylindrical conidia. But, in *Cylindro-*

cladium the spores are produced singly at the apices of sterigmata while in the present genus the spores are produced successively in great abundance forming slimy balls measuring up to 100 μ in diameter and consisting of hundreds of conidia. Secondly, the general pattern of conidiophore and especially the shape and structure of the spore-bearing branches (phialides) are quite different in the two genera. In *Cylindrocladium* the metulae are distinct and bear two or three short swollen sterigmata with tapering ends, while in the present fungus the phialides are cylindrical and almost undifferentiated, and the terminal portions are successively cut off into long cylindrical conidia. Besides these important differences, there is a total absence of chlamydospores, sclerotia and the special torch-shaped hyphae with swollen ends, so typically and consistently described for *Cylindrocladium* by various authors. Attention may also be drawn here to another genus, *Candelospora*, (type species *C. ilicicola* Hawley, 1912) which resembles *Cylindrocladium* closely. It has similar conidiophores with multiseptate conidia. Recently Boedijn and Reitsma (**1950**) have reviewed all the species of *Cylindrocladium*, including species of *Candelospora*. These authors regard *Candelospora* synonymous with *Cylindrocladium*, with which it agrees in all details excepting degree of septation in conidia. The cultural characteristics of the present fungus are also markedly different from the descriptions given for species of *Cylindrocladium*.

SUMMARY

A new genus of Moniliaceae is described. The fungus resembles *Penicillium* in the structure of the conidial apparatus and *Gliocladium* in the formation of balls of conidia embedded in slime at the apices of the conidiophores. But this genus differs radically from these two genera in having very thin cylindrical spores which are 2-celled.

The cultural characteristics have been recorded on a number of culture media. Affinities have been briefly discussed.

ACKNOWLEDGMENTS

The writer is very grateful to Dr. R. K. Saksena for his kind guidance and advice and to Dr. Charles Thom for very kindly going through the description with the diagrams. His advice has been very valuable. His thanks are also due to Prof. Fr. H. Santapau for the Latin diagnosis and to Dr. R. Misra for his kindly encouragement.

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LITERATURE CITED

Boedijn, K. B. and J. Reitsma. 1950. Notes on the genus *Cylindrocladium*. Reinwardtia **1**: 51–60.

Clements, F. E. and C. L. Shear. 1931. The genera of fungi. New York.

Fawcett, H. S. and L. J. Klotz. 1937. A new species of *Candelospora* causing decay of citrus fruits. Mycologia **29**: 207–215.

Gilman, J. C. 1944. Manual of soil fungi. Ames.

Maerz, A. and M. R. Paul. 1950. A dictionary of color. New York.

Massey, L. M. 1917. The crown canker disease of rose. Phytopathology **7**: 408–417.

Raper, K. B. and C. Thom. 1949. A manual of the Penicillia. Baltimore.

Waksman, S. A. 1927. Principles of soil microbiology. Baltimore.

——. 1952. Soil microbiology. New York.

IMMOBILIZATION OF FUNGUS SPORES AND OTHER MINUTE OBJECTS IN WATER MOUNTS

HARRY K. PHINNEY [1] AND JOHN R. HARDISON [2, 3]

(WITH 2 FIGURES)

The microscopic examination of minute biological objects mounted in liquids presents a number of problems. Foremost among these are: (1) brownian movement and drift of the objects, (2) disposition of the objects in several planes in the field, and (3) movement of the objects as a result of the displacement of the coverslip by the viscous immersion fluid. Any movement is annoying during detailed examination or measurement but becomes especially troublesome when photographs are to be taken. Disposition of the objects in the field in more than one plane will always make difficult the preparation of good photomicrographs. Most low viscosity water base mounting media have an objectionably low refractive index and in addition will float the cover glass after it is pressed down, thus pulling small objects out of a single plane of alignment.

We were very kindly presented with a sample of sodium carboxy methyl cellulose (NaCMC) by Mr. A. M. Scott of the New Orleans Academy of Science who recounted the value of its gelling properties in the preparation of a simple aqueous medium which allowed the control of orientation of cells of the Desmideaceae by movement of the cover glass during observation. This material has been recommended by several authors for inclusion in permanent mounting media of complex composition which require additional handling of the material to prepare it for observation. Such media are most successful with chitinous or woody objects impervious to shrinkage and plasmolysis.[4]

[1] Department of Botany and Plant Pathology, Oregon State College.

[2] Division of Forage Crops and Diseases, U. S. Department of Agriculture and Oregon Agricultural Experiment Station.

[3] Approved for publication as Technical Paper No. 812 by the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Botany and Plant Pathology.

[4] Clark, Edgar W. and Frank Morishita. C-M Medium: A mounting medium for small insects, mites, and other whole mounts. Science **112**: 789–790. 1950.

The material now being used in this laboratory is NaCMC grade CW-72, lot type 70.[5] It has been found most expedient to add the dry powder in sufficient quantity to obtain a suspension of the required viscosity directly to the water suspension of spores or other small cells on the microscope slide. This allows for proper dispersal of the cells without the inclusion of large numbers of air bubbles in the mount. Application of the dry powder directly to the mount on the slide also obviates storage of the suspension which is liable to bacterial and fungal deterioration. It is likewise advantageous in that the amount of NaCMC can be varied to meet the demand of the particular material under observation. In thicker suspensions some algal and fungal cells show plasmolysis and distortion; usually, a thinner suspension will avoid this difficulty.

A suitable gel for a great many objects is given by a suspension of approximately 12 percent which has a refractive index of 1.3418.[6] It has the property of stopping pedesis and movement of the coverslip during oil immersion examination. By the simple action of applying pressure to the cover glass with a sliding motion it is possible to insure the orientation of all objects in the mount in one plane. When the spores are only 1 or 2 μ in width, a minimum of the mounting medium of thinner consistency and considerable pressure to the cover glass are desirable; of course, it is impossible to achieve the desired results in the presence of extraneous material larger than the spores. For these very small objects, it is sometimes advantageous to use less than a 12 percent initial suspension by applying the chemical evenly in small quantities to the water until the gel is first recognized. Immediate addition of the cover glass and application of pressure produces a thinner layer of medium more easily than starting with a heavier gel. Evaporation of a part of the water from the medium will then produce the consistency required for immobilization.

Disposition of the minute (3–4 × 4–6 μ) spores of *Ustilago hypodytes* (Schlect.) Fries in a single plane is illustrated by FIG. 1. Measurement of minute spores from such 1000 × photographs with a millimeter ruler is facilitated when a maximum number of spores is in median optical section. This orientation is even more helpful with short, cylindrical spores, such as the small (5–6 × 1.5–2 μ) pycnidiospores of *Phoma lingam* (Fr.) Desm., many of which invariably will be situated in many undesired axial positions in most other temporary mounting media.

[5] Supplied by E. I. duPont de Nemours & Company, Inc., Wilmington 28, Dela.

[6] The assistance of Mr. Duis Bolinger of the Physics Department, Oregon State College, in determination of the refractive index is gratefully acknowledged.

Elimination of these difficulties is demonstrated by FIG. 2 showing the nearly single plane alignment of the minute pycnidiospores of *P. lingam*.

If spore walls are decorated in some distinctive fashion, a photograph of a mount showing spores at various levels may be useful. Such photographs, however, are unpleasant to study, and it is difficult to make measurements from them. It would appear that using the present technique, it would be preferable to present two photographs, one a surface

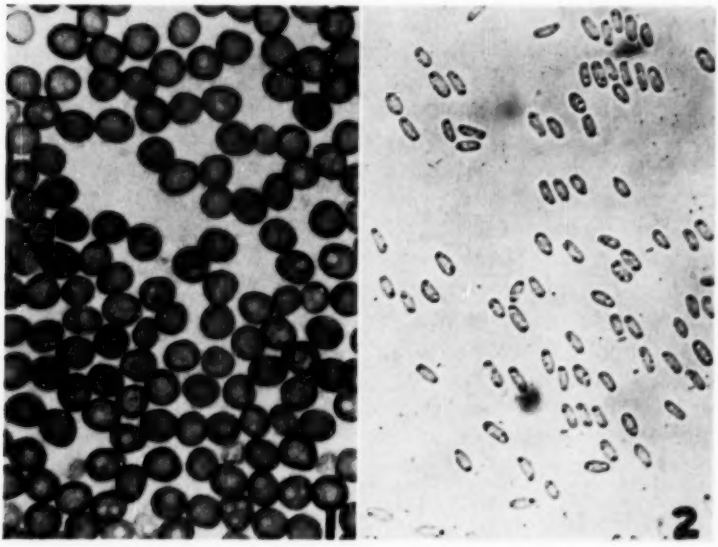

FIG. 1. Spores of *Ustilago hypodytes* from *Agropyron repens*, × 1000.
FIG. 2. Pycnidiospores of *Phoma lingam*, × 1000.

view to show wall decoration and one a median section for mensuration and to show another view of wall modifications.

NaCMC and other methyl cellulose compounds are marketed under various proprietary names. Some of these compounds may have many and perhaps all of the requisite features for the preparation of similar satisfactory media.

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NOTES AND BRIEF ARTICLES

The 1950 Foray of the Mycological Society of America

The Foray was held in the Medicine Bow Mountains, Wyoming, Aug. 7–10, with the University of Wyoming Science Camp serving as headquarters. Cabins were provided for single parties and for family groups. The central lodge was available during the day for those not participating in field trips, and for the comfort of all after field trips were over and specimens laid out to dry. Meals were served in the cafeteria of the Lodge. A less expensive and, at the same time, more enjoyable foray could hardly be envisioned. The Society deeply appreciates the courtesies extended to it by the University of Wyoming through Dean O. C. Schwiering, College of Education, and Mr. S. H. Knight, Director of the Science Camp. Members who attended the foray will long remember the considerate attention given to their comfort by Mr. Everett Lantz, the Camp's efficient business manager.

The scientific pursuits of the Foray were organized by Dr. W. G. Solheim, Professor of Botany at the University of Wyoming. The well-appointed botanical laboratory at the Science Camp was made available as a center for collecting activities. Microscopes and dryers were provided for our use, and the laboratory's library of literature pertaining to the botany and mycology of the region proved invaluable. The success of the Foray resulted in large measure from Dr. and Mrs. Solheim's knowledge of the region based upon personal experience, and from the active participation of Dr. Alexander H. Smith, President of the Mycological Society, who spent the summer of 1950 collecting agarics and other fungi in the Medicine Bow Mountains.

Front row: (kneeling and seated) Ronald E. Binegar, The Shaw Children, Erdman West, Nancy Smith, The Henry Children, Mrs. Alexopoulos.

Second row: (kneeling) A. H. Smith, W. G. Solheim, Mrs. Cooke, Phyllis Gardner MacMillan, Mrs. John Baxter, Miss Sally Couch, L. K. Henry, Mrs. Henry.

Third row: (standing) L. J. Wickerham, Wm. Bridge Cooke, Mrs. Solheim, Dr. Franz Petrak, J. F. Brenckle, P. Duffield, Mrs. Shaw, Charles Gardner Shaw, John Baxter, Lee Bonar, Mrs. Couch, John N. Couch, Mrs. Meisness, Gail Fosler, C. J. Alexopoulos, Mrs. Raper, Mrs. West, E. S. Beneke, Mrs. Smith.

Also present at the Foray but not represented in the photo: J. C. Gilman, R. W. Davidson, D. Burgloff, V. Wilson, Mrs. L. H. Tiffany, G. W. Fischer, R. Sprague, Josef Zoril, Kenneth B. Raper.

FIG. 1. THE FORAY GROUP. (Explanation on opposite page.)

The principal region in which the Foray was held is described and illustrated in: Oosting, H. J. and J. F. Reed. "Virgin Spruce-Fir Forest in the Medicine Bow Mountains, Wyoming." Ecological Monographs **22**: 69–91. 1952. Two ranges, Pole Mountain and The Snowy Range, in the Medicine Bow Mountains, in southeastern Wyoming, were the sites of several collecting trips, as follows:

Pole Mountain, east of Laramie. Lodgepole pine forest, 8000 to 9000 feet.

Science Camp environs. Spruce-fir forest, about 9000 ft.

North Fork of the Little Laramie River. Lodgepole pine woods with spruce, about 8300 ft.

Tanner Lake, in The Snowy Range. In upper spruce-fir forest and timberline, 10,000–12,000 ft.

Headquarters Park, west side of the Snowy Range, spruce-fir forest, about 10,500 ft.

Informal evening programs were presented in the lobby of the Lodge during the Foray, each being illustrated by slides in natural color. Dr. Solheim gave us a botanical and ecological tour of the Medicine Bow region. President Alexander H. Smith, University of Michigan, discussed the fleshy fungi of the western mountains and the scenery of the Cascade Range. Professor Erdman West, Plant Pathologist at the University of Florida, Gainesville, presented scenes and plants of Florida. One of the writers (W.B.C.) showed scenes and plants of Mount Shasta, California, while the other (K.B.R.) discussed the ubiquitous genus *Penicillium*.

Two social events of the Foray will long be remembered. These included the fish-fry on the shores of Lake Marie and the luncheon at the Solheims. Prior to the Foray, Drs. Smith and Solheim spent the better part of two weeks in the invigorating mountain air making collections not in woodlands with vasculum and mushroom basket, but along lakeshores with rod and fly. These skilled collectors proved equally adept at cooking piscatorial specimens, a fact amply verified by the accompanying group picture of the happy, well-fed mycologists made immediately after the memorable fish-fry on Wednesday, Aug. 9. A view of the scenery around Lake Marie in the Snowy Range is also presented.

On our return from the Pole Mountain trip Thursday we were entertained at luncheon at the home of Dr. and Mrs. Solheim in Laramie. This most enjoyable experience served as a fitting climax to an altogether delightful and rewarding foray.

Our most distinguished visitor was Dr. Franz Petrak (retired), of the Vienna Natural History Museum, editor of *Sydowia*, who, although

FIG. 2. Lake Marie, scene of the Fish-Fry. Grovesand individual trees of *Abies lasiocarpa*, Subalpine Fir, and thickets of huckleberry are evident in the foreground. To the left and beyond the lake is The Snowy Range.

not in good health, enjoyed the opportunity of collecting flowering plants for the Herbarium at Vienna which had been wantonly destroyed during the Second World War. He was brought to the Foray by an old friend and correspondent, Dr. J. F. Brenckle of North Dakota.

The Society was fortunte in being able to have a foray in the higher mountains of western United States. As a result, many members had the opportunity of studying in the field an interesting series of fungi with which they do not usually come in contact. Especially interesting from an ecological viewpoint were the fungi associated with the timberline and with the trees and flowering plants which make up the strictly montane to subalpine vegetation. Several interesting range extensions were recorded: *Hydnotria variiformis* Gilkey is the first truffle reported for Wyoming. It was found first by Dr. Alexopoulos, and subsequently a mature specimen was found by Ronald E. Binegar in a rotten log. It is a timberline truffle previously reported from Mount Shasta, California, and several Oregon Coast Range peaks. A second timberline fungus previously reported only from Cascade Range peaks was *Calvatia fumosa* Zeller, which was fairly common.

The accompanying list of fungi was compiled from lists returned by the collectors listed below. Species names are followed by the initials of the collectors who reported them. Collectors' names are followed by the names of the institutions, or herbaria, in which their foray material is deposited. C. J. Alexopoulos (Michigan State College); J. W. Baxter (Herbarium of J. W. Baxter); R. E. Binegar (Herbarium of H. M. Gilkey, Washington State College); Lee Bonar and Phyllis G. MacMillan (University of California); W. B. and V. G. Cooke (Washington State College, Dept. of Plant Pathology); G. W. Fischer (Washington State College, Dept. of Plant Pathology); Kenneth B. Raper (Northern Regional Research Laboratory); C. G. Shaw (Washington State College, Dept. of Plant Pathology); A. H. Smith (University Herbarium, University of Michigan); W. G. Solheim (University of Wyoming); R. Sprague (Washington State College, Dept. of Plant Pathology); L. J. Wickerham (Northern Regional Research Laboratory); F—Specimens exhibited at the Foray some of which were not retained.

LIST OF SPECIES REPORTED

MYXOMYCETES: **Acrasiales**—*Dictyostelium mucoroides* Brefeld (KBR). **Myxogasteres**—*Arcyria cinerea* (Bull.) Pers. (CJA); *A. denudata* (L.) Wettst. (CJA); *A. incarnata* Pers. (CJA); *A. versicolor* Phill. (CJA); *Comatricha nigra* (Pers.) Schroet. (CJA); *Dictydium cancellatum* (Batsch.) Macbr. (CJA);

Diderma floriforme (Bull.) Pers. (CJA); *D. globosum* Pers. (CJA); *D.* sp. (CJA); *Fuligo septica* (L.) Web. (CJA); *Hemitrichia clavata* (Pers.) Rost. (CJA); *Lycogala epidendrum* (L.) Fr. (CJA); *Physarum luteolum* Pk. (CJA); *Trichia varia* Pers. (CJA).

PHYCOMYCETES: *Synchytrium aureum* Schroet. on *Viola* sp. (LB, CGS); *S.* spp. on *Plantago tweedyi* (LB), *Polygonum bistortoides* (CGS), *Pentstemon* sp. (CGS), *Sibbaldia procumbens* (CGS), *Veronica alpina* (CGS), *Draba verna* (CGS), *Saxifraga arguta* (CGS); *Physoderma pluriannulatum* (B. & C.) Karling on *Angelica grayi* (LB); *Peronospora parasitica* (Pers.) Fr. on *Draba verna* (CGS); *P. trifoliorum* DBy. on *Melilotus officinalis* (CGS); *Mortierella* sp. in soil (KBR).

ASCOMYCETES: **Saccharomycetaceae**—*Hansenula canadensis* Wickerham on dead upright *Pinus contorta* var. *latifolia* and wet grass (LJW); *H. capsulata* Wickerham on live *Picea engelmannii*, dead *Pinus contorta* var. *latifolia* (LJW); *H. minuta* Wickerham in bog water (LJW); *H. suaveolens* (Klöcker) Dekker in cow dung (LJW). **Erysiphaceae**—*Erysiphe communis* Wallr. ex Fr. (*Oidium* stage) on *Achillea millefolium* (CGS); *E. graminis* DC. ex Mérat on *Agropyron trachycaulon, Poa alpina, Poa secunda, Puccinellia nuttalliana* (GWF & RS); *Sphaerotheca macularis* var. *fuliginea* (Schlecht. ex Fr.) W. B. Cke. on *Pedicularis racemosa* (CGS). **Hypocreaceae**—*Hypomyces aurantius* (Pers. ex Fr.) Tul. on *Polyporus versicolor, P. ursinus* (WBC); *H. papyraceus* (Ell. & Holw.) Seaver on *Picea engelmannii* (WBC). **Phyllachoraceae**—*Phyllachora junci* (Fr.) Fckl. on *Juncus vaseyi* (CGS). **Lophostomataceae**—*Lophionema apoclastospora* Solh. on *Salix* sp. (WGS). **Mycosphaerellaceae**—*Mycosphaerella punctiformis* var. *clematidis* on *Epilobium* sp. (CGS); *M. tassiana* (DeNot.) Johanss. on *Senecio triangularis* (WBC). **Pseudosphaeriaceae**—*Didymosphaeria clematidis* on *Clematis ligusticifolia* (CGS); *Herpotrichia nigra* Hartig on *Abies lasiocarpa* (LB); *H. quinqueseptata* Weir on *Picea engelmannii* (WBC); *Leptosphaeria erigerontis* Berl. on *Agroseris aurantiacus, Delphinium reticulatum* (CGS); *Metasphaeria sepalorum* Vleugel on *Juncus parryi* (WBC); *Trichosphaeria solaris* (Cke. & Ell.) Ell. & Ev. on *Picea engelmannii* (WBC); *Zignoella diaphana* var. *soluta* (Ell. & Ev.) Ell. & Ev. on conifer wood (LB). **Hypodermataceae**—*Bifusella acuminata* (Ell. & Ev.) Bonar & W. B. Cke. on *Juncus parryi* (WBC); *Hypodermella abietis-concoloris* (Mayr) Dearn. on *Abies lasiocarpa* (LB); *Lophodermium arundinaceum* (Schrad. ex Fr.) Chev. on *Oryzopsis exigua, Poa alpina, Poa fendleriana, Poa secunda* (RS); *Lophodermina juniperina* (Fr.) Tehon on *Juniperus communis* var. *siberica* (CGS, LB). **Patellariaceae**—*Tympanis pinastri* Tul. on conifer (F). **Helotiaceae**—*Dasyscypha agassizii* (Berk. & Curt.) Curt. on *Picea engelmannii, Abies lasiocarpa* (LB, WBC); *D. arida* (Phill.) Sacc. on *Picea engelmannii, Abies lasiocarpa* (LB, WBC); *Lachnum bicolor* (Bull.) Karst. on *Populus tremuloides* (LB). **Sclerotiniaceae**—*Sclerotinia bifrons* Seaver and Shope (*Sclerotium* stage) on *Populus tremuloides* (CGS, LB). **Pezizaceae**—*Paxina acetabulum* (L. ex Fr.) O. Kuntze on the ground (WBC); *Peziza sylvestris* (Boud.) Sacc. & Trott. on the ground (F); *Pseudoplectania nigrella* (Pers.) Fckl. on the ground (WBC). **Tuberaceae**—*Hydnotria variiformis* Gilkey in rotten conifer logs (CJA, REB).

BASIDIOMYCETES: HETEROBASIDIOMYCETES: **Pucciniales — Melampsoraceae**—*Chrysomyxa arctostaphyli* Diet., III, on *Arctostaphylos uva-ursi* (JWB); *C. pyrolae* (DC.) Rostr., I, II, on *Pyrola secunda* (CGS, JWB), *Pyrola* sp. (CGS);

Cronartium comandrae Pk., II, III, on *Comandra pallida* (JWB); *Coleosporium solidaginis* (Schw.) Thuem., I, on *Erigeron peregrinus* var. *callianthemus* (CGS); *Hyalopsora polypodii* (Pers.) Magn., II, on *Cystopteris fragilis* (LB, WBC); *Melampsora albertensis* Arth., II, on *Populus tremuloides* (JWB); *M. ribesii-purpurae* Kleb., II, on *Salix planifolia* (JWB); *Melampsorella cerastii* (Pers.) Schroet., O, I, on *Picea engelmannii* (LB, JWB, WBC); *Milesia darkeri* Faull, II, on *Cryptogramma acrostichoides* (LB, JWB); *Pucciniastrum goeppertianum* (Kühn.) Kleb., III, on *Vaccinium membranaceum* (WBC); *P. pyrolae* (Pers.) Schroet., II, on *Pyrola secunda* (CGS). **Pucciniaceae**—*Cumminsiella mirabilissima* (Pk.) Nannfeldt, II, on *Berberis repens* (CGS, JWB); *Phragmidium andersonii* Shear, III, on *Potentilla fruticosa* (JWB); *P. ivesiae* Syd., III, on *Potentilla subjuga, P. gracilis* (JWB); *P. rosae-acicularis* Liro, III, on *Rosa* sp. (JWB); *P. rosae-arkansanae* Diet., III, on *Rosa ?fendleri* (CGS); *P. rubi-idaei* (DC.) Karst., III, on *Rubus strigosus* (JWB); *Puccinia absinthii* (Hedw. f.) DC., III, on *Artemisia aromatica* (JWB), II, III, on *Artemisia tridentata* (JWB); *P. albulensis* Magn., III, on *Veronica subalpina* (JWB); *P. arnicalis* Pk., II, III, on *Arnica cordifolia* (JWB); *P. atrofusca* (D. & T.) Holw., I, on *Artemisia cana* (JWB), II, III, on *Carex geyeri* (JWB); *P. balsamorhizae* Pk., III, on *Balsamorhiza sagittata* (LB); *P. bistortae* (Str.) DC., II, III, on *Polygonum bistortoides* (LB, JWB, WBC), on *Polygonum viviparum* (JWB); *P. calochorti* Pk., III, on *Calochortus gunnisonii* (JWB); *P. caricis-grossulariata* Arth., I, on *Ribes saxosum* (JWB); *P. cirsii* Lasch, III, on *Cirsium* sp. (JWB); *P. caricis-shepherdiae* J. J. Davis, I, on *Shepherdia canadensis*, II, on *Carex aquatilis* (JWB); *P. coronata* Cda., O, I, on *Shepherdia canadensis* (LB, CGS, WBC); *P. coronata* var. *bromi* F. & L., III, on *Bromus ciliatus* (JWB); *P. coronata* var. *calamagrostis* F. & L., II, III, on *Calamagrostis koeleroides* (JWB); *P. crandallii* Pam. & Hume, I, on *Symphoricarpos albus* (JWB); *P. expansa* Lk., III, on *Senecio* sp. (JWB); *P. extensicola* var. *valerianae* Arth., I, on *Valeriana furfurescens* (JWB); *P. gentianae* (Str.) Lk., III, on *Gentiana bigelovii* (JWB); *P. grindeliae* Pk., III, on *Chrysopsis fulcrata* (JWB); *P. heucherae* (Schw.) Diet., III, on *Saxifraga arguta* (CGS); *P. hieracii* (Schum.) Mart., I, II, III, on *Agoseris glauca* (CGS, JWB); *Agoseris aurantiaca, Taraxacum officinale* (CGS); *P. jonesii* Pk. var. *jonesii*, III, on *Lomatium macrocarpum* (CGS); *P. ligustici* Ell. & Ev., III, on *Ligusticum* sp. (JWB), on *Angelica grayi* (LB); *P. menthae* Pers., III, on *Mentha arvensis* (JWB); *P. monoica* (Pk.) Arth., II, III, on *Koeleria cristata, Trisetum spicatum* (RS); *P. palmeri* Diet. & Holw., I, III, on *Pentstemon whippleanus* (JWB); *P. poae-sudeticae* (Westd.) Jørstad, II, III, on *Poa* sp. (JWB), *Poa alpina* (RS), *Poa nervosa* (RS), *Poa palustris* (RS), *Trisetum spicatum* (JWB); *P. polygoni-amphibii* Pers., III, on *Polygonum* sp. (JWB); *P. pseudocymopteri* Holw., II, III, on *Pseudocymopterus* sp. (JWB); *P. rubigo-vera* var. *agropyri* (Erikss.) Arth., III, on *Agropyron* sp. (JWB), I, on *Aquilegia coerulea* (JWB), *Thalictrum* sp. (JWB, WBC); *P. rubigo-vera* var. *apocrypta* (Ell. & Tr.) Arth., I, on *Phacelia heterophylla* (JWB); *P. stipae* Arth., III, on *Stipa comata* (JWB); *P. treleasiana* Paz., III, on *Caltha rotundifolia* (CGS, JWB); *P. vagans* var. *epilobii-tetragoni* DC., I, on *Epilobium adenocaulon* (JWB), III, on *Epilobium perplexans* (JWB); *P. violae* (Schum.) DC., III, on *Viola* sp. (LB, CGS); *Uromyces aemulus* Arth., III, on *Allium brevistylum* (JWB); *U. heterodermus* Syd., O, III, on *Erythronium grandiflorum* (LB, JWB, WBC); *U. intricatus* Cke., II, on *Eriogonum umbellatum* (JWB); *U. jonesii* Pk., III, on *Ranunculus calthae-*

florus (JWB); *U. junci* (Desm.) L. Tul., I, on *Grindelia erecta* (JWB); *U. lapponicus* Lagerh., I, III, on *Oxytropus albiflorus* (JWB); *U. minor* Schroet., III, on *Trifolium parryi* (CGS); *U. punctatus* Schroet., III, on *Oxytropus gracilis* (JWB, WBC); *U. trifolii* (Hedw.) Lev., O, I, on *Trifolium parryi* (CGS, LB, JWB); *U. zygadeni* Pk., I, on *Zygadenus elegans* (JWB). **Ustilaginales—Ustilaginaceae**—*Ustilago jacksonii* Zundel & Dunlap on *Stipa lettermani* (GWF); *U. striiformis* (Westend.) Niessl on *Agrostis alba, Danthonia intermedia, Poa secunda* (GWF). **Tilletiaceae**—*Entyloma arnicale* Ell. & Ev. on *Arnica cordifolia* (CGS), *Arnica latifolia* (CGS); *E. compositarum* Farl. on *Erigeron peregrinus* var. *callianthemis* (with *Ramularia* conidial stage) (CGS); *Tilletia cerebrina* Ell. & Ev. on *Deschampsia caespitosa* (GWF); *T. elymi* Diet. & Holw. on *Elymus glaucus* Buckl. (GWF); *Urocystis agropyri* (Preuss) Schroet., on *Agropyron subsecundum, Agropyron trachycaulum, Phleum alpinum, Poa nervosa, Trisetum spicatum* (GWF). **Tremellales—Dacrymycetaceae**—*Dacrymyces deliquescens* (Bull.) Duby. on wood (LB); *Guepiniopsis alpinus* (Tr. & Earle) Brasfield on *Picea engelmannii* (LB, WBC).

HOMOBASIDIOMYCETES: **Exobasidiales—Exobasidiaceae**—*Exobasidium vaccinii* (Fckl.) Wor. on *Arctostaphylos uva-ursi* (CGS, WBC), *Vaccinium membranaceum* (CGS). **Aphyllophorales—Thelephoraceae**—*Coniophora umbrina* Alb. & Schw. ex Fr. on conifer wood (WBC); *Corticium corruge* (Burt) Burt in sched. ex Rogers & Jackson on *Picea engelmannii*, and on assorted substrata near melting snow including bark of any nearby tree, litter of all kinds, and stones (LB, WBC, WGS); *Cytidia salicina* (Fr.) Burt on *Salix* sp. (WBC, WGS); *Pellicularia subcoronata* (Hoehn. & Litsch.) Rogers on conifer wood (WBC); *Peniophora byssoides* (Pers. ex Fr.) Bres. on rotting log, humus, rooting and charred conifer wood (WGS); *Solenia anomala* (Pers. ex Fr.) Fckl. on *Salix* sp. (WBC); *Stereum hirsutum* Willd. ex Fr. on *Populus tremuloides* (WBC); *S. rufum* Fr. on *Populus tremuloides* (CGS, LB, WBC); *S. rugisporum* (Ell. & Ev.) Burt on *Abies lasiocarpa, Picea engelmannii* (CGS, LB, WBC); *S. sanguinolentum* Alb. & Schw. ex Fr. on *Picea engelmannii* (WBC); *Thelephora caryophyllea* Schaeff. ex Fr. on the ground (WBC); *Tomentella ferruginosa* (Hoehn. & Litsch.) Sacc. & Trott. on soil under log (WGS); *Vararia granulosa* (Fr.) Laurila on rotting wood (WGS). **Hydnaceae**—*Dentinum repandum* (L. ex Fr.) S. F. Gray on the ground (WBC); *Odontia alutacea* (Fr.) Bres. on rotting wood (CGS). **Clavariaceae**—*Clavaria flava* var. *aurea* Coker on the ground (WBC). **Polyporaceae**—*Cryptoporus volvatus* (Pk.) Hubbard on *Pinus contorta* var. *latifolia* (WBC); *Fomes igniarius* (Fr.) M. C. Cooke on *Populus tremuloides* (LB, WBC); *F. nigrolimitatus* (Romell) Egeland on *Picea engelmannii, Pinus contorta* var. *latifolia* (WBC); *F. pini* (Thore ex Fr.) Lloyd on *Pinus contorta* var. *latifolia* (LB); *Ganoderma applanatum* (Fr.) Pat. on *Populus tremuloides* (specimen of rot only) (WBC); *Lenzites sepiaria* Wulf. ex Fr. on *Picea engelmannii* (WBC); *Polyporus adustus* Willd. ex Fr. on *Populus tremuloides* (WBC); *P. alboluteus* Ell. & Ev. on *Picea engelmannii* (LB, WBC); *P. elegans* Bull. ex Fr. on sticks in litter (WBC); *P. leucospongia* Cke. & Harkn. on *Picea engelmannii* (WBC); *P. perennis* L. ex Fr. on litter and duff (WBC); *P. picipes* Fr. on litter (WBC); *P. ursinus* Lloyd on *Picea engelmannii* (LB, WBC); *Poria xantha* (Fr.) M. C. Cooke on *Populus tremuloides* (WBC). **Agaricales—Hygrophoraceae**—*Hygrophorus erubescens* (Fr.) Fr. (AHS); *Hygrocybe conicus* Scop. ex Fr. (F). **Tricholomataceae**—*Armillaria albolanaripes* Atk. (AHS, F); *Armillariella mellea*

(Vahl. ex Fr.) Karst. (AHS, F); *Clitocybe odora* (Fr.) Kummer (AHS, F); *Collybia cookii* (Bres.) J. D. Arnold (WBC, F); *C. dryophila* (Bull. ex Fr.) Quél. (F); *C. maculata* (Fr.) Kummer (AHS, F); *C. nitellina* (Fr.) Quél. (AHS, F); *Flammulina velutipes* (Curt. ex Fr.) Singer (F); *Laccaria laccata* (Fr.) Berk. & Br. (AHS, F); *Lentinus omphalodes* Fr. (AHS, F); *Lepista personata* (Fr. ex Fr.) W. G. Smith (AHS, F); *Lyophyllum* sp. (WBC); *Marasmiellus fibulus* (Bull. ex Fr.) Singer (F); *Marasmius epiphyllus* (Pers. ex Fr.) Fr. (AHS, F); *Mycena pura* (Pers. ex Fr.) Quél. (F); *M. thujina* A. H. Smith (AHS); *Pleurotus ostreatus* (Jacq. ex Fr.) Quél. (F); *Tricholoma flavovirens* (Fr.) Lundell (AHS, F); *T. imbricatum* (Fr.) Quél. (AHS, F); *Xeromphalina campanella* (Batsch. ex Fr.) Kuehn. & Maire (F). **Amanitaceae**—*Amanita inaurata* Secr. (F); *A. muscaria* (L. ex Fr.) Pers. ex S. F. Gray (F). **Agaricaceae**—*Lepiota clypeolaria* (Fr.) Kummer (AHS, F). **Coprinaceae**—*Coprinus atramentarius* (Bull. ex Fr.) Fr. (F); *C. micaceus* (Bull. ex Fr.) Fr. (F); *Panaeolus campanulatus* (Fr.) Quél. (AHS, F); *Psathyrella spadicea* (Fr.) A. H. Smith (AHS). **Strophariaceae**—*Kuehneromyces vernalis* (Fr.) Singer & Smith (F); *Pholiota squarrosa* (Pers. ex Fr.) Quél. (F); *P. spumosa* (Fr.) Singer (AHS, F); *Psilocybe coprophila* (Fr.) Kummer (AHS); *Stropharia semiglobata* (Fr.) Quél. (AHS). **Cortinariaceae**—*Alnicola melinoides* (Fr.) Kuehner (F); *Cortinarius brunneus* Fr. (AHS, F); *C. cinnamomeus* Fr. (AHS); *C. collinitus* (Pers. ex Fr.) Fr. (F); *C. crassus* Fr. (AHS, F); *C. glaucopus* Fr. (AHS, F); *C. laniger* Fr. (F); *C. multiformis* Fr. (F); *C. primosus* Pk. (F); *Hebeloma crustuliniforme* (Bull. ex Fr.) Quél. (F); *Inocybe armoricana* Heim (AHS); *I. calamistrata* (Fr.) Gill. (F); *I. geophylla* (Sow. ex Fr.) Quél. var. *geophylla* (F); *I. geophylla* var. *lilacina* (Pk.) Boudier (F); *I. subdecurrens* Ell. & Ev. (F). **Rhodophyllaceae**—*Rhodophyllus byssisedus* (Fr.) Quél. (AHS). **Gomphidiaceae**—*Gomphidius rutilus* (Schaeff. ex Fr.) Lundell & Nannfeldt (WBC, AHS, F). **Boletaceae**—*Boletus edulis* Bull. ex Fr. (F); *Leccinum aurantiacum* (Bull. ex Fr.) S. F. Gray (F); *Suillus americanus* (Pk.) Snell (F); *S. brevipes* (Pk.) O. Kuntze (AHS, F); *S. granulatus* (L. ex Fr.) O. Kuntze (F); *S. luteus* (L. ex Fr.) S. F. Gray (AHS, WGS, F); *S. tomentosus* (Kauffman) Singer (AHS, F). **Russulaceae**—*Lactarius aspideus* (Fr. ex Fr.) Fr. (F); *L. controversus* (Pers. ex Fr.) Fr. (AHS, F); *L. deliciosus* (L. ex Fr.) S. F. Gray (AHS, F); *L. torminosus* (Schaeff. ex Fr.) S. F. Gray (F); *L. uvidus* (Fr. ex Fr.) Fr. (F); *Russula delica* Fr. (F); *R. densifolia* (Secr.) Gill. (AHS, F); *R. emetica* (Schaeff. ex Fr.) Pers. ex Fr. (AHS); *R. fallax* (Fr.) Sacc.-sensu Singer (AHS, F).

GASTEROMYCETES: **Lycoperdales—Lycoperdaceae**—*Bovista plumbea* Pers. (F); *Calvatia cyathiformis* (Bosc.) Morg. (WBC); *C. fumosa* Zeller (WBC) (F); *Lycoperdon perlatum* Pers. (WBC, F); *L. pyriforme* Pers. (WBC, F).

FUNGI IMPERFECTI: **Phomales—Phomaceae**—*Ascochyta sorghi* Sacc. on *Agropyron trachycaulon* (RS); *Darluca filum* (Biv.) Cast. on rusts on *Poa alpina, Poa nervosa, Poa palustris, Trisetum spicatum* (RS); *Diplodia* sp. on *Purshia tridentata* (fragmentary, no species listed on this host) (CGS); *Phaeoseptoria poae* Sprague on *Poa ampla* (RS); *Phoma pedicularis* Wehmeyer on *Pedicularis racemosa* (CGS); *P. herbarum* Westd. on *Arenaria fendleri* (CGS); *Phomatospora therophila* (Desm.) Sacc. on *Carex* sp. (CGS); *Phyllosticta arnicae* Fckl. on *Arnica cordifolia* (CGS, LB); *P. brunellae* Ell. & Ev. on *Prunella vulgaris* (CGS);

P. erythronii W. B. Cke. & C. G. Shaw on *Erythronium parviflorum* (CGS); *P. ferax* Ell. & Ev. on *Lupinus* spp. (CGS); *P. trollii* Trail on *Trollius albiflorus* (LB); *P.* sp. on *Agroseris aurantiaca* (CGS); *Selenophoma donacis* (Pass.) Sprague & A. G. Johnson on *Elymus glaucus* (RS); *S. donacis* var. *stomaticola* (Baumel.) Sprague & A. G. Johnson on *Agropyron griffithsii* (RS); *S. everhartii* (Sacc. & Syd.) Sprague & A. G. Johnson on *Deschampsia atropurpurea* (RS); *Septoria avenae* Frank on *Glyceria grandis* (RS); *S. calamagrostidis* (Lib.) Sacc. on *Trisetum montanum* (RS); *S. heraclei* (Lib.) Desm. on *Heracleum lanatum* (CGS); *S. nodorum* Berk. on *Elymus glaucus* (RS); *S. osmorhizae* Pk. on *Osmorhiza chilensis* (CGS). **Leptostromataceae**—*Heteropatella umbillicata* (Pers.) Jaap on *Juncus parryi* (WBC); *Leptostromella conigena* Dearn. on *Picea engelmannii* (cones) (CGS). **Melanconiales—Melanconiaceae**—*Hainesia borealis* Ell. & Ev. on *Galium boreale* (CGS, WBC); *Marssonina populi* (Lib.) Magn. on *Populus tremuloides* (LB); *Phleospora osmorhizae* (Pk.) House on *Osmorhiza obtusa* (LB). **Moniliales—Moniliaceae**—*Acrostalagmus* sp. in soil (KBR); *Aspergillus fumigatus* Fres. in soil (KBR); *A. terreus* Thom in soil (KBR); *Bactridium flavum* Kze. & Schum. on wood (WBC); *Cephalosporium* sp. in soil (KBR); *Cylindrosporium geranii* Ell. & Ev. on *Geranium caespitosum* (CGS); *C. heraclei* Ell. & Ev. on *Heracleum lanatum* (LB); *C. montanegrinum* Bubák on *Trollius albiflorus* (LB); *C. ranunculi* (Bon.) Sacc. on *Ranunculus macounii* (CGS); *Penicillium brevi-compactum* Dierck in soil (KBR); *P. canescens* Sopp in soil (KBR); *P. crustosum* Thom in soil (KBR); *P. cyclopium* Westling in soil (KBR); *P. daleae* Zaleski in soil (KBR); *P. fellutanum* Biourge in soil (KBR); *P. frequentens* Westling in soil (KBR); *P. granulatum* Bainier in soil (CJA); *P. kapuscinskii* Zaleski in soil (KBR); *P. lanoso-coeruleum* Thom in soil (KBR); *P. lanoso-griseum* Thom in soil (KBR); *P. lanosum* Westling in soil (KBR); *P. lilacinum* Thom in soil (KBR); *P. lividum* Westling in soil (KBR); *P. miczynskii* Zaleski in soil (KBR); *P. palitans* Westling in soil (KBR); *P.* (near) *pulvillorum* Turfitt (KBR) in soil; *P.* (near) *raistrickii* Smith (KBR) in soil; *P. solitum* Westling in soil (KBR); *P.* (near) *terlikowskii* Zaleski in soil (KBR); *P. terrestre* Jensen in soil (KBR); *P.* (near) *velutinum* van Beyma in soil (KBR); *Ramularia castillejae* Ell. & Ev. on *Castilleja* sp. (CGS); *R. cercosporioides* Ell. & Ev. on *Epilobium* sp. (CGS); *R. senecionis* (B. & Br.) Sacc. on *Senecio triangularis* (CGS); *R. tulasnei* Sacc. on *Fragaria americana* (LB, CGS); *Rhynchosporium orthosporum* Caldwell on *Agropyron subsecundum* (RS); *Scolecotrichum graminis* Fckl. on *Agropyron spicatum, Agropyron subsecundum, Calamagrostis canadensis, Danthonia intermedia, Elymus glaucus, Festuca kingii, Glyceria pauciflora, Phleum alpinum, Phleum pratense, Poa alpina, Poa secunda* (RS); *Spicaria* sp. in soil (KBR). **Dematiaceae**—*Arthrinium bicorne* Rostrup on *Juncus parryi* (WBC); *Cercospora montana* (Speg.) Sacc. on *Epilobium angustifolium* (LB); *C. sii* Ell. & Ev. on *?Sium cicutaefolium* (CGS); *Cladosporium herbarum* Lk. ex Fr. on *Pinus flexilis* (CGS); *Heterosporium allii* Ell. & Mart. on *Allium cernuum* (CGS); *H. phlei* Gregory on *Phleum alpinum* (RS); *Septogloeum oxysporum* Sacc., Bomm. & Rouss. on *Agrostis alba* (RS), *Agrostis thurberiana* (LB). **Tuberculariaceae**—*Fusarium avenaceum* (Fr.) Sacc. on *Puccinia poae-sudeticae* on *Poa alpina* (RS).—KENNETH B. RAPER, VICE PRESIDENT, UNIVERSITY OF WISCONSIN, MADISON, WIS., WM. BRIDGE COOKE, RECORDER, ROBERT A. TAFT SANITARY ENGINEERING CENTER, CINCINNATI, OHIO.

A FURTHER REPORT ON THE SOIL STORAGE OF FUNGI

The author [1] recently reported on the use of loam as a storage medium for fungi. The 18 species stored in it were divided into two groups: A with 5 species and B with 13. He found that 4 species in A and 13 in B were viable after one year and that 2 of the fungi in A were viable even after 4 years. On loam, all the viable fungi remained in their "wild" or primary state, whereas on agar many of them did not. For example on agar the dermatophytes, and also *Phoma*, *Phyllosticta* and *Fusarium*, became pleomorphic in 1–2 months.

In the present investigation 94 stock cultures of other fungi were stored in loam, with agar cultures as controls. Now, after an interval of two years, this paper reports on the viability and cultural stability of these fungi. It also reports further on the 17 fungi in groups A and B kept on loam for 2½ to 5½ years.

FUNGI STORED IN LOAM FOR TWO YEARS

Absidia cylindrospora, **Allescheria boydii*, **Anthostomella destruens*, *Aspergillus* (2 species), *A. fumigatus*, *A. niger*, * *Blastomyces brasiliensis* (2 isolates), *Candida* sp., * *Choanephora* sp., * *Chromocrea spinulosa* (6 isolates), *Circinella* sp., *C. minor*, * *Cladosporium* sp., *Coccospora agricola*, *Crytococcus neoformans* (2 isolates), *Cunninghamella elegans*, *Fimetaria fimicola*, *Geotrichum* (2 species), *G. candidum*, * *Gelasinospora autosteria*, *G. cerealis*, * *G. adjuncta*, *G. calsopora*, *G. retispora*, *G. tetrasperma* (5 isolates), *Hansenula anomala*, * *Haplosporangium parvum* (14 isolates), *Hormodendrum compactum*, **H. pedrosoi*, * *Lichtheimia corymbifera*, * *Microsporum canis* var. *album*, *Monilia* sp., *Monosporium apiospermum*, * *Mortierella isabellina*, *M. reticulata*, *Maccromucor* sp., * *Mucor microsporus*, *M. racemosus*, *M. varians*, **Neurospora sitophila* (4 isolates), *N. tetrasperma*, *Paecilomyces* sp., *Penicillium* sp., *Phialophora verrucosa* (3 isolates), *Phycomyces* sp. (2 strains), * *Pichia* sp., *Pleurage tetrasperma*, *Rhizopus nigricans* (2 isolates), *Sacchoromyces cerevisiae*, *Scopulariopsis brevicaulis*, * *Sepedonium* sp., * *Sporobolomyces* sp., *Syncephalastrum racemosum*, * *Thamnidium elegans*, * *Trichophyton verrucosum* (3 isolates), *T. rubrum* (3 isolates), *Zygorhynchus* sp.

It was found that on agar the controls of the 4 fungi in A were dead when examined at 5½ years and, with the exception of *N. asteroides*,

[1] Bakerspigel, A. Soils as a storage medium for fungi. Mycologia **45**: 596–604, 1953.

* Some of these cultures on agar were found to be dead at two years.

all the fungi in group B were dead at 2½ years. Of the remaining 94 fungi on agar, 64 were viable at 2 years while the 30 cultures of those fungi marked with an asterisk had died. Viable cultures of *Rhizopus* and *Zygorhynchus* on agar were composed of white mats of mycelium devoid of their characteristic sporangia while *Aspergillus* and *Penicillium* had white tufts of sterile mycelium arising at several points on their surfaces.

The viability and cultural stability of all the fungi stored in loam were tested by removing a small quantity of loam with a sterile loop and streaking it on plates of agar.

In loam all the fungi (except *M. audouini* and *C. albicans* in group B) were viable and in their "wild" state.

This report therefore confirms earlier findings that loam is a useful storage medium for fungi, since in it most of the species can be preserved in their "wild" state from 2½ to 5½ years.—A. BAKERSPIGEL, PROVINCIAL LABORATORY OF PUBLIC HEALTH, UNIVERSITY OF ALBERTA, EDMONTON, ALBERTA.

SIMPLIFIED TECHNIQUE FOR GROWING FUNGI IN SLIDE CULTURE

All current methods for growing fungi in slide cultures are based on the principle of incubation in a moist chamber. Sterile slides or cover glasses are placed on a layer of filter paper in a petri dish. A drop of melted culture medium is placed on the slide or cover slip and inoculated. The filter paper is moistened in order to maintain the required humidity. The chamber is then incubated until the required stage of growth has been reached.

There are several modifications of this basic method.[1, 2, 3, 4] While yielding beautiful results, they all require elaborate preparations in the way of sterilization of the chambers, of pipettes and of water. The manipulations necessary for placing medium, for inoculation, moistening, etc., require repeated opening and closing of the chamber, with correspondingly increasing danger of contamination. Lastly, the small quan-

[1] Lewis & Hopper, Int. Med. Mycol. ed. 3. 1948.

[2] Skinner, Emmons & Tsuchiya, Henrici's Molds, Yeasts, and Actinomycetes, ed. 2. 1948.

[3] Riddell, R. W., Mycologia **42**: 265, 1950.

[4] Rivalier and Seydel, in Langeron and Vanbreuseghem, Précis de Mycologie, p. 416. Paris, 1952.

tity of culture medium may dry out before a representative stage of growth is reached, unless more moisture is added during incubation.

The procedure described below reduces preparation and manipulation to a minimum, while yielding good results. The following materials are required:

1. Plates with solid culture medium (any desired medium may be used).
2. One dish with melted culture medium containing 0.75% to 1% agar only.
3. Forceps.
4. Cover slips, 16 mm square.

The procedure is as follows:

1. With flamed forceps grasp cover slip at one corner and flame. Allow the cover slip to cool for a moment.
2. Dip the cover slip into the melted culture medium and withdraw. A thin film of medium will adhere to the cover slip and solidify almost immediately.

FIG. 1. *Hormodendrum pedrosoi.* Slide culture according to method described in text.

3. Deposit the coated cover slip on the surface of a dish containing solid culture medium. Up to four cover slips may be placed on the same surface, but care should be taken that they do not touch or overlap.
4. Place a pinpoint inoculum in the center of a coated cover slip.
5. Seal and invert the plate and incubate.

The progress of growth may be checked under the low power lens of the microscope. When the desired stage of development has been reached, a few drops of 95% alcohol are placed on the cover slip cultures and allowed to evaporate. The cover slips are then carefully removed from the plates and inverted into a drop of lactophenol cotton blue on a clean slide. More dye may be added, or excess removed with filter paper, as necessary. The thin film of culture medium does not interfere with the clarity of the preparations, as shown in FIG. 1.—CLAIRE L. TASCHDJIAN, DEPARTMENT OF DERMATOLOGY AND SYPHILOLOGY, NEW YORK UNIVERSITY POST-GRADUATE MEDICAL SCHOOL (DR. MARION B. SULZBERGER, CHAIRMAN) AND THE SKIN AND CANCER UNIT OF NEW YORK UNIVERSITY HOSPITAL.

ON OVERHOLTS' CONSERVATISM

In 1911, L. O. Overholts published his first list of species of polypores found in Ohio. This list was based on collections made by him and records found by him. It was greatly influenced by Murrill, as a casual glance at the nomenclature will easily show. The work was done during Overholts' junior year at Miami University, Oxford, Ohio. Three years later, from Burt's laboratory at the Missouri Botanical Garden he published an extended list with descriptions and notes for all species known at that time to occur in Ohio. During the intervening three years Overholts changed his ideas about nomenclature. He gives no reason in the second paper for his withdrawal from the Murrill system. It is entirely possible that some notes by C. G. Lloyd may be helpful.

In Mycological Writings **4**: Letter 55, pages 1 and 2, published in March, 1915, we find an item headed "Overholts' Polyporaceae of Ohio." A few quotations from this may be of interest: "There appeared about one year ago in the Annals of the Missouri Botanical Garden (March, 1914), a paper by L. O. Overholts on the Polyporaceae of Ohio. We have seen notices of the paper, but as a separate issue was not sent to me, and as I was not favorably impressed with a previous paper by the same author, I did not take the trouble to go over to the library and look it up. It has therefore really just come to my atten-

tion and I was agreeably surprised to find it to be a very valuable and true presentation of the subject, and I am gratified that Mr. Overholts has gotten back into the realms of rational mycology. . . . The present paper I consider the best and most valuable paper on the classification of the native Polyporaceae that has yet been printed. . . . With these few exceptions, I believe Overholts' paper is strictly correct, and, as the truth, will finally prevail. It is the only paper that has yet appeared on American polypores that is reasonably accurate, and the only paper of much value."

Since Overholts mentioned in the first paper that he had made considerable use of the Lloyd Library and since he indicated that he had had assistance with identification from C. G. Lloyd it is easy to see that Mr. Lloyd would have been very greatly distressed with the first effort. It is quite probable that Mr. Lloyd made some sort of attempt to express his opinion either directly or indirectly to the young student. Overholts, probably being quite impressionable, took the caustic comments to heart and after changing schools, from the influence of Dr. Bruce Fink to that of Dr. Burt, was further encouraged to become more conservative.

D. P. Rogers reports that in a letter from Dr. Burt to Dr. Farlow, filed at the Farlow Herbarium, Dr. Burt asked Dr. Farlow's opinion of the adoption of Murrill's genera. The reply by Dr. Farlow is not on file but we may assume that it discouraged the use of Murrill's genera and encouraged the conservative system Overholts used after he left Miami University.

The conservatism expressed in the second Ohio paper was continued throughout the remaining 30 years of his active life. In fact, if one follows the series of papers from the second Ohio paper through the Midwestern polypore paper to his Pennsylvania series and finally the recently published book, it will be seen that with the exception of the addition of species, the polishing of descriptions, the extension of habitat and geographic range, and the development of additional notes for many of the species there is little difference between the members of the series. In fact, the keys and their arrangement differ little among the several papers.

The following words, written in June, 1916, thirty years before Overholts' death, are as applicable now to the Overholts book: "It is extremely gratifying to me to be able to give strong approval to a work issued on mycology. It is an excellent work. It is the first comprehensive and reasonably accurate account that has been given of the subject. Mr. Overholts has selected his names, in most instances, I think,

with good judgment, and practically all are of established usage. There are a few of his names that are, according to my ideas, not meritorious, but compared with the great bulk of the work, they are not worth quibbling over." From a notice on "Polyporaceae of the middle-western United States" by C. G. Lloyd, Mycological Writings **5**: 586, June, 1916.

That Overholts took such comments to heart is evidenced by a statement commending Lloyd's work found on page 8 of the introduction to the new book: "The work of Lloyd was avowedly conservative, and extremely lucid and inclusive."

Dr. Overholts' views concerning the breaking up of *Polyporus* and *Fomes* are stated as follows: "But to follow any principle of procedure logically and consistently in the matter of recognizing generic segregates in *Polyporus* and *Fomes* leads either to results that have no significance in a natural scheme of classification, or else to the point where the conception of genera encroaches too closely upon that of species. The result in the first instance would in no way be an improvement over existing conditions, and in the second case the main principle underlying our present method of classifying plants is set at naught" (pp. 6–7 of the Introduction).

For several paragraphs Overholts then goes on to discuss the several methods of splitting the genera of polypores which had been proposed up to the time he finished working on the manuscript, ending with a comment on the system proposed by Bondarzew and Singer. On pages 9 and 10 he comments as follows on the writer's proposal, since adopted by Bessey in his textbook: "Cooke does not propose any new generic concepts, but apparently takes the attitude that if a name can be shown to be valid under the International Rules we are obligated to use that name, regardless of the need for it." Of course, this is an extreme interpretation of the situation, for in the last of the several lists of genera given in Cooke's paper there are a number of genera which could have been added to those presented in the key but which were not used because of possible relationships with other genera. Some of these genera will eventually be incorporated in keys; some of those used in the key will be discarded.

Finally, in reference to splitting, Overholts says (p. 9): "Bourdot and Galzin have presented the polypores of France in a very lucid and understanding account, and one which appeals to me as having much merit. They recognize about twenty genera, mostly in the sense of their compatriots Quélet and Patouillard. For those who insist that it is time for a radical revision of the polypores, yet who dislike the small and monotypic genera of other authors, this scheme is recommended."

Nothing is said of the species which do not occur in France but which, if the scheme is followed consistently, must be included in "small and monotypic" genera.

Beginning in the fall of 1931, and more intensively from the fall of 1935 through the spring of 1937, the writer collected polypores and other fungi in a number of locations throughout southern Ohio. Representatives of many of these collections were sent to Dr. Overholts for identification or confirmation of identification, reminiscent of his own technique of some twenty years before. During the course of our correspondence, the writer asked certain pertinent questions for his own information concerning Dr. Overholts' ideas of phylogeny and taxonomy of the polypores. In reply to certain of these questions the following statements were made in a letter dated Dec. 4, 1936.

"As to the family Boletaceae, I use the genus *Boletus* in the old sense, although perfectly cognizant of the fact that Europeans usually recognize a number of generic segregates. As in all cases where systematic work is being done, there is a tendency to split up the old into many or several units. It is a matter of opinion as to which is the desirable method; if you are trained as a splitter, then you will not be satisfied unless you split. I see no justification in so much splitting.

"For me the *Boleti* constitute a family entirely distinct from the Polyporaceae, having taken their origin from certain gill fungi in the neighborhood of *Paxillus*, and have no relationships with any of the Polyporaceae.

"In the *Polyporaceae* I place *Polyporus, Poria, Fomes, Daedalea, Lenzites, Trametes, Favolus, Cyclomyces, Solenia,* and *Hexagona.* Eventually I may recognize the genus *Ganoderma,* merely because the trend is in that direction at present. *Porothelium* I do not regard as a good genus, but its species referable to *Poria.*

"If the family *Meruliaceae* is recognized I would be inclined to include in it the genus *Phlebia.* Still, it is merely an intermediate genus and fits about as well one place as another.

"The trouble is, that there are so many intermediates any way you limit families that you cannot escape them entirely. A re-arrangement of the whole Basidiomycete scheme is in the offing, although it will not be worked out satisfactorily in my time. At the present time our families are delimited along horizontal lines that represent levels of development attained. But these stages or levels were many times reached by different lines of development. There is no better representative of this example than in the Boletes and the Polypores. Because they both developed tubes they were thrown together into one

family. Yet there is little question among mycologists today that the tube condition of the Boletes was reached by a transverse partitioning of the gills of *Paxillus*-like ancestors while the ancestry of the Polypores lies in the *Thelephoraceae* through certain primitive *Hydnaceae* and *Merulius*. *Solenias* probably arose from *Cyphella*-like ancestors; *Clavaria* from *Craterellus*-like forms which ancestry also produced at least a part of the present agaric flora. Likewise *Hydnum* and the whole series of *Hydnaceae* arose from a *Thelephoraceous* ancestry, the bulk of resupinate *Hydnaceae* from *Corticium*-like ancestors. In fact, I look upon the simpler Thelephoraceae as the group from which have originated perhaps all of the Hymenomycetous lines of development either directly or indirectly. I doubt that *Lenzites* has an agaricaceous ancestry, developed into a plant of tougher consistency in response to xerophytic conditions. For me it and *Daedalea* do not represent transition forms between polypores and agarics. It's a beautiful theory and there are nice intergrading forms. But *Daedalea* has indirectly a Thelephoraceous ancestry through the polypores and in turn is the group ancestral to *Lenzites*—entirely on a distinct line of development than the Agaricaceae. *Irpex* is not to be derived from irpiciform species of *Polyporus* such as *P. tulipiferae*, or if it is, then *Irpex* belongs to the *Polyporaceae*, lock, stock and barrel, and is in no wise related to the Hydnaceae, but has merely shown a development that parallels that in the Hydnaceae. Probably *Irpex* combines at present species of two lines of development—one that is distinctly Hydnaceous and the other Polyporaceous. For that reason I have removed from that genus two species, *tulipiferae* and *farinacea*, in which the evidence is relatively clear, into the Polyporaceae. Perhaps all of them should be so classed; I haven't seen enough early stages in development to know, but I am inclined to think that *I. crassus* (= *I. mollis*), and perhaps others, are true hydnaceous plants, with an ancestry identical with that of other *Hydnaceae*."

"Well, I have writen too much. Don't worry too much about your classification. It will all be out of date in another 50 years anyway. We're due for a great shake-up, but it will come gradually, and the result will be a more natural, though less practical, arrangement."

It appears that Dr. Overholts was still waiting for the more natural but less practical rearrangement of the Hymenomycetes when he finished reorganizing his manuscript. It also appears that he was not satisfied with any of the present (at the time of his last revision) systems. It would be interesting to see the type of rearrangement of which he would have approved—whether it would continue to follow the

horizontal levels he described or whether it would cut across these levels at one or more points. If any system were not outdated in fifty years it would indicate that the ultimate had been achieved and in the Hymenomycetes this utopian situation is hard to conceive because of the various avenues of approach, which have hardly been started upon at this time. In the meantime those who dare to propose new rearrangements of the species involved at the generic level must be willing to see their systems blasted time and again by other innovators who are also trying to bring order out of chaos.—WM. BRIDGE COOKE, ROBERT A. TAFT SANITARY ENGINEERING CENTER, CINCINNATI 26, OHIO.

REVIEWS

LES GENRES SORDARIA ET PLEURAGE, LEURS AFFINITÉS SYSTÉMATIQUES, by Claude Moreau. (Encyclopédie Mycologique, Vol. XXV) 330 pp., 79 figs. Paul Lèchevalier, Paris, 1953 (1954). Price 3500 fcs. (about $10.00).

In this work on *Sordaria*, *Pleurage*, and associated genera, Claude Moreau has recognized that the aim of taxonomy must be a synthesis of data on all aspects of the fungi and, as a consequence, has produced what might properly be termed a biologic study. In addition to the systematic treatment, there are sections dealing with the morphology, cytology, physiology, genetics, and ecology of fungi in *Sordaria* and *Pleurage*. After a historical review of systems of classification of the Ascomycetes, Moreau attempts a more logical distribution of the genera which, largely on the basis of habit and superficial resemblance, have been assembled in the Sordariaceae. The family Sordariaceae is maintained in the Sphaeriales for *Sordaria*, *Pleurage*, *Bombardia*, *Triangularia*, *Gelasinospora*, *Neurospora*, and *Coniochaeta*. *Hypocopra* is placed in the Xylariaceae, while *Delitschia*, *Sporormia*, and *Pleophragmia* are removed to the Pseudosphaeriales.

Although extensive data are presented on other genera, only *Sordaria* and *Pleurage* receive detailed systematic study. Moreau includes in *Sordaria* only four species which he considers well defined. All species in which the ascospores are provided with either primary or secondary appendages are placed in *Pleurage*, which is chosen over *Podospora* on the basis of debatable priority. In fact, two species lacking primary appendages, *Sordaria leporina* Cain and *S. ontariensis* Cain, are placed in synonymy with *Pleurage setosa* (Winter) Kuntze, a species in which primary appendages are present. Moreau lists a number of correlated characters in support of this separation of *Pleurage* and *Sordaria*. The most important of these is ascus structure, the ascus tip in *Sordaria* being provided with an annular ring, which is lacking in *Pleurage*. The correlation is not perfect. Ascus rings are present in two species, *Pleurage neglecta* (Hansen) Cl. Moreau and *P. inaequilateralis* (Cain) Cl. Moreau, which lack primary appendages. This might be interpreted as evidence favorable to the hypothesis that the secondary appendages are of doubtful significance and that the separation of these genera

should be based on the presence or absence of primary appendages. *Pleurage* contains the majority of the species, although the number in this genus is reduced, chiefly by placing forms with multiple-spored asci in synonymy with the corresponding forms with eight-spored asci.

Moreau's casual disregard for rules of nomenclature will doubtless prove disturbing to a number of taxonomists. When species with multiple-spored asci and those with eight-spored asci are reduced to synonymy, the name of the eight-spored species is used, regardless of priority. No Latin diagnoses are provided for the new species *Sporormia petasoniformis* Cl. Moreau and the new genus *Bombardioidea* Cl. Moreau.

Genetics and ecology are given only token consideration. The sections on cytology deal almost entirely with the vacuolar system and offer little on the behavior of the nuclei in relation to the life cycle. The sections on physiology deal primarily with cultural characteristics, the results being employed in grouping the species of *Pleurage* into sections. They also provide some interesting data on factors affecting the production of perithecia, especially on interaction between organisms in mixed cultures. The conclusion that addition of fragments of filter paper stimulates perithecium formation in *Pleurage* spp. by altering the texture of the medium might be questioned. Morphology receives the most extensive treatment. Moreau emphasizes the taxonomic importance of structure of the "nucleus" (centrum) of the perithecium, ascus structure, and method of expulsion of the ascospores. The detailed descriptions and illustrations of ascus structure in all genera are excellent. Although there are extensive descriptions of the gross morphology of the mycelium and perithecia and data on the origin of the perithecia in *Sordaria* and *Pleurage*, there is practically nothing on the development of the centrum in any of the genera studied. This is particularly disappointing since the data on this phase of development in these genera are fragmentary and contradictory, and, as Moreau states, centrum structure is of fundamental importance in determining taxonomic relationships.—E. S. LUTTRELL.

CORRECTED PAGE

from *A. arbuscula* and E chromosomes from *A. javanicus* var. *macrogynus* that occur as a result of haphazard chromosome distribution at meiosis in the F_1. Occasional recovery of pure E or H progeny is likewise understandable. Variation in other characters such as sex ratio and pigmentation can also be explained. That each individual F_1 gametophyte when selfed then ordinarily transmits its particular combination of gametangial arrangement and other characteristics uniformly

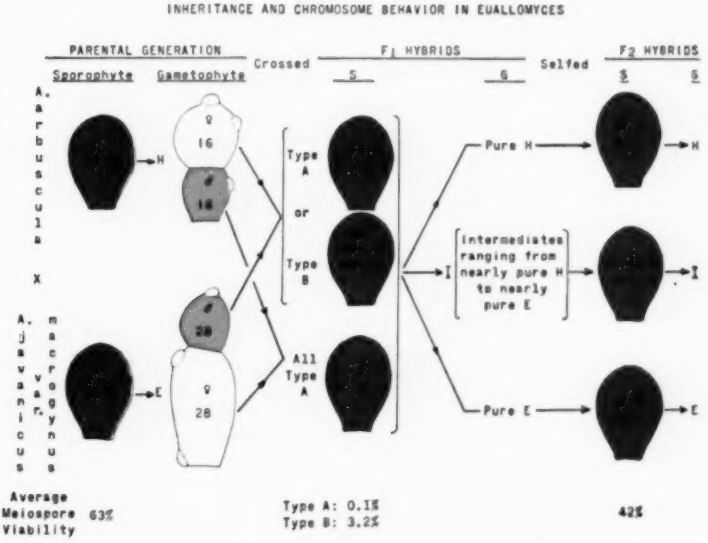

FIG. 7. Chart correlating the genetics and chromosome cytology of interspecific reciprocal hybrids in *Euallomyces*. Male gametangia are indicated by the orange color; female gametangia are colorless; meiosporangia are brown. The numbers within these structures show the chromosome counts and the extent of pairing at meiosis. The lettering is as follows: S, sporophyte; G, gametophyte; H, hypogyny; E, epigyny; A and B, the two types of nuclei in F_1 hybrids (see text).

to its offspring follows from the regular pairing of the identical chromosome sets at meiosis in the F_2 and subsequent generations. The chart shown in FIG. 7 is designed to summarize the main features of chromosome number and behavior in the artificial hybrids. Further discussion of the inheritance of epigyny and hypogyny, as well as an interpretation of the occasional sudden mutations that occur in the F_2 and F_3, will be presented in the following sections.

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